Circulation
The Journal of the American Heart Association
JULY 1951
VOL. IV NO. 1

Abscesses of the Valve Rings of the Heart, a Frequent but Not Well Recognized Complication of Acute Bacterial Endocarditis

By WALTER H. SHELDON, M.D., AND ABNER GOLDEN, M.D.

Abscesses of the valve rings of the heart were encountered at autopsy in 86 per cent of patients with acute bacterial endocarditis who had received antibiotic therapy. The abscesses, which appeared to arise from mycotic aneurysms in the valve rings, represented in most instances the only persistent focus of acute inflammation. They may account in part for the unsatisfactory results in the treatment of acute bacterial endocarditis, and their frequent occurrence suggests a relationship to antibiotic therapy.

SINCE 1947 we have encountered 12 cases of acute bacterial endocarditis in which single or multiple abscesses of the valve rings of the heart were present. Abscesses were found in 86 per cent of all cases of acute bacterial endocarditis which came to autopsy during this period. The lesions, although of appreciable size, were not conspicuous on routine examination of the heart; in fact, they were easily overlooked if not specifically sought. We believe that the abscesses represent a not well recognized complication of acute bacterial endocarditis. We have failed to find similar lesions in 11 cases of subacute bacterial endocarditis during the same period. The valve ring abscesses may be of clinical significance and their frequent occurrence might be related to therapy with antibiotics.

METHOD

We first noted abscesses in a case of acute bacterial endocarditis which at autopsy showed marked involvement of the aortic valve together with a single mass of vegetation on the anterior mitral cusp. The gross appearance of the vegetation was not unusual, considering that the patient had received extensive penicillin therapy, but it was noted that the vegetations of the anterior mitral cusp arose directly over the valve ring and did not involve the free edge of this valve. Close inspection and palpation of the valve ring suggested an ill-defined swelling which could also be felt to involve the ring of the adjacent aortic leaflet. Upon incision a cavity was found which exuded purulent material and which was partially filled with laminated blood clot. Abscesses were specifically sought in all subsequent cases of acute and subacute bacterial endocarditis and the following method of dissection was evolved.

The heart was opened in the usual manner and the vegetations cultured. All postmortem clots were removed and the valve rings were carefully inspected. In the later cases areas suspicious of abscess were aspirated and the contents cultured. All four valves were then photographed. Following the usual examination of the heart, the entire heart skeleton was dissected in one block. This specimen included all valves, as well as a strip of myocardium and mural endocardium of both atria and ventricles adjacent to the valve ring.

The entire specimen was then fixed in Zenker's fluid with 5 per cent glacial acetic acid. After fixation, the valves, valve rings and adjacent myocardium were sectioned at 2 to 3 mm. intervals. In this manner, any grossly recognizable lesion involving the valve rings was visualized in its entire extent and could be photographed.

From the Departments of Pathology, Emory University School of Medicine, Grady Memorial Hospital and Emory University Hospital, Atlanta, Ga.

Presented at the Annual Meeting of the Southern Section of the American Federation for Clinical Research in New Orleans, Louisiana on March 17, 1950.

Multiple blocks were taken from each valve ring. All blocks included the valve, valve ring and adjacent myocardium. Many, often subserial, sections were cut and stained with hematoxylin and phloxine, phloxine-methylene blue, Wilder's modification of the reticulum stain, Weigert's elastic tissue stain, Gram stain and occasionally, Mallory's aniline blue stain. In addition, multiple sections representing all four chambers of the heart were examined.

The other organs were dissected in appropriate manner and examined both grossly and histologically.

Autopsy Findings

The description to follow represents a summary of the autopsy findings of all 12 patients. The findings with which we are chiefly concerned were similar in all cases, and for this reason it is felt that a single case report suffices for illustration.

M. J., a 59 year old white woman, was hospitalized on Feb. 4, 1949. She had been in fairly good health until three weeks before admission when she developed a persistent nonproductive cough. One day later she experienced the first of many severe chills. Her family stated that she had been feverish throughout her illness. Two days before admission she became irrational.

It was thought that the patient had had valvular heart disease for many years, but no history of acute rheumatic fever could be obtained. She had experienced numerous syncopal attacks during early adult life. Orthopnea and exertional dyspnea had been present during the two years before admission.

Physical Examination. The temperature was 104.6 F., the pulse rate 108 and the respiratory rate 22 per minute. The blood pressure was 96/64. The patient appeared acutely ill and was irrational. The neck veins were moderately distended. Moist rales were heard over both lung bases. The apex impulse of the heart was located 11.5 cm. to the left in the fifth intercostal space. The cardiac rhythm was regular. A harsh grade III systolic murmur was audible along the entire left border of the heart; no diastolic murmurs were heard. There was a minimal pitting edema of the lower extremities.

Laboratory Data. The urine specific gravity was 1.016 and there was 1 plus albuminuria. The urinary sediment was not abnormal. A serologic test for syphilis was negative. The red blood cell count was 3.5 million per cu. mm. and the blood hemoglobin concentration was 8.8 Gm. per 100 cc. The erythrocyte sedimentation rate was 85 mm. in one hour (Westergren). The white blood cell count was 8750 per cu. mm., later rising to 20,150. The blood nonprotein nitrogen concentration on admission was 38 mg. per 100 cc., but later rose to 148. Lumbar punctures done on two occasions revealed normal findings. Electrocardiograms showed left bundle branch block and later during her illness auricular fibrillation.

Seven blood cultures drawn at the time of admission grew pneumococcus type 32. These organisms grew in 0.01 unit but not 0.02 unit per cc. of penicillin. Four blood cultures taken on the eighth day of penicillin treatment were sterile.

Hospital Course. The patient was given 100,000 units of aqueous penicillin intramuscularly every three hours for 12 days, receiving a total of 9.7 million units. Her temperature remained elevated varying between 99 and 101 F. On the third hospital day she was digitalized and then maintained on digitoxin. On the sixth day it was noted that the systolic murmur heard at the time of admission was now barely audible, while a loud high pitched diastolic murmur was present over the primary and secondary aortic areas.

The patient showed little response to therapy and died on the thirteenth day of hospitalization. Signs of congestive heart failure were minimal at the time of death.

Gross Pathologic Findings. The heart weighed 550 Gm. and the pericardium was covered by a fibrinous exudate. The valve measurements were: tricuspid 12.1 cm., pulmonary 8.3 cm., mitral 9.8 cm., aortic 7.3 cm. The left ventricle measured 2 cm. in thickness and the right 0.6 cm.

The left atrium contained an unattached antemortem thrombus measuring 2 by 2 by 1 cm. The annulus of the anterior mitral cusp showed a glistening, red and friable vegetation, 1.8 by 1.5 cm. (fig. 1*C*). Several nodular areas of calcification measuring 3 to 4 mm. were located on the ventricular surface of the annulus of the posterior cusp.

The left leaflet of the aortic valve was covered with innumerable, minute, shiny, friable, yellow-gray vegetations involving the ventricular surface, part of the free edge and the sinus of Valsalva (fig. 1*A*). The free edge of this leaflet was rolled. An oblique 1.5 cm. tear extended from the commissure connecting this and the posterior leaflet, into the valve as far as the annulus. Just beneath this leaflet was an ulceration, 2.0 by 1.2 cm., covered by reddish-brown soft tissue. On cut surface the ulceration led into a cavity, 1.3 by 0.7 cm., containing soft reddish brown material (fig. 2*A*). The cavity extended upward behind the root of the aorta, where it appeared to involve the myocardium of the left atrium and downward into the annulus of the anterior mitral cusp.

The right aortic leaflet was also covered by many shiny yellowish-gray vegetations ranging from 1 to 4 mm. in size (fig. 1*A*). They involved the ventricular surface and the sinus of Valsalva, but spared the thickened free edge. In the center of the leaflet near its left side was an out-pouching, 6 by 2 mm.

Just below the leaflet was an ulceration, 3.5 by 1.2 cm., containing soft grayish-red material. The fundus of the sinus of Valsalva showed an 8 mm. cone shaped retraction with a 2 mm. central opening. Cut surface showed a cavity which measured 1.7 by 0.6 cm. and contained soft grayish-red material (fig. 2*B*). The cavity extended upward for a short distance behind the root of the aorta and involved the commissure between the right and posterior aortic leaflets. It also extended into the annulus of the right and left leaflets of the pulmonic valve.

The two cavities located respectively behind the right and left aortic leaflet did not communicate with each other.

The posterior aortic leaflet including its sinus of Valsalva was almost completely calcified, but revealed no vegetations (fig. 1*A*).

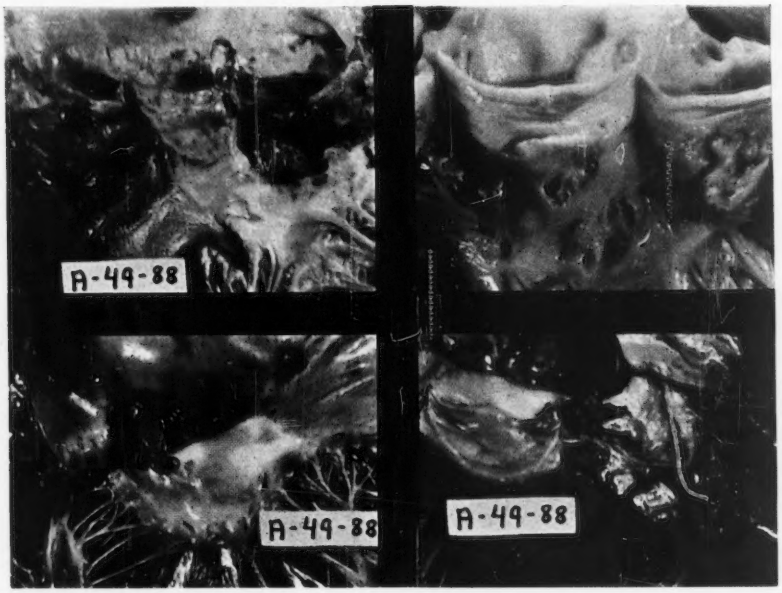


FIG. 1*A* (top left). Acute bacterial endocarditis of aortic valve. The left leaflet (at right) is partly destroyed and covered by vegetations extending to the commissure, into the sinus of Valsalva and to the ventricular aspect of the anterior mitral cusp. The opening at the base of the valve leads into the abscess shown in figure 2*A*. The posterior leaflet (center) is thickened. The free edge of the right leaflet (shown on the left) although thickened, is uninvolved by vegetations which cover the remainder of this structure and extend to the interventricular septum.

B (top right). Aortic valve with syphilitic aortitis and valvulitis. Multiple crater-like openings on mural endocardium beneath posterior leaflet (left) represent points of rupture of underlying valve ring abscess associated with acute bacterial endocarditis of mitral valve. The left leaflet (right) shows near its base a ruptured valvular aneurysm. Note that the free edge of both leaflets shows no vegetations.

C (bottom left). Anterior mitral cusp with healing vegetation attached to valve ring. The vegetation is secondary to an abscess involving the aortic and mitral ring shown in figure 2*A*. Note that remainder of valve is grossly normal.

D (bottom right). Atypically located healing vegetations on commissure between left and right pulmonic valve leaflets. The vegetations overlie an abscess extending from the aortic and the pulmonic ring shown in figure 2*B*. The abscess is associated with acute bacterial endocarditis of the aortic valve. Note that the vegetations arise from the sinuses of Valsalva and the mural endocardium beneath the commissure but do not involve the leaflets.

The pulmonic valve showed three separate yellowish-brown vegetations (fig. 1*D*). One measured 8 by 9 mm. and arose from the mural endocardium just beneath the commissure between the right and left leaflets. The others arose from the same commissure in the sinus of Valsalva of the right leaflet, measured about 5 by 3 mm. each, and did not

involve the valve leaflet. The remaining portions of the pulmonic valve were not remarkable. Cut surfaces showed that the vegetations overlay the above described cavity behind the root of the aorta (fig. 2*B*).

The tricuspid valve and the remaining portions of the mural endocardium were not remarkable.

The myocardium displayed multiple minute scars and the papillary muscles and trabeculae of the left ventricle showed indistinct zigzag yellow lines. The coronary arteries revealed diffuse atheromatous plaques without gross occlusion.

The aorta showed some atherosclerosis, but the coronary orifices were only slightly encroached upon. Small old and recent infarcts were present in the lungs, spleen, kidneys and in the right cerebellar hemisphere. Additional gross findings were chronic passive congestion of the liver, a pedunculated polyp measuring 4 by 2 cm. in the sigmoid, obliteration of the appendix, and fibrous adhesions around the cecum and in the right pleura.

Cultures taken at autopsy yielded gamma streptococcus and *Pseudomonas aeruginosa* from the heart blood, coagulase negative *Staphylococcus aureus* from the spleen and no growth from the lung.

Histologic Findings. All aortic leaflets showed extensive old fibrosis and focal calcification. The right and left leaflets showed many areas of necrosis and were covered on both surfaces by vegetations. The necrotic areas and the vegetations showed generally advanced repair with endothelialization and organization (fig. 3). The mitral valve revealed old fibrosis with minimal focal calcification. Areas of necrosis of the valve and vegetations were seen on the anterior cusp. The latter were present on the ventricular surface where they represented an extension of the lesion of the left aortic leaflet. Another mass of vegetations was present on the atrial surface at the base of the cusp. The necrotic areas and vegetations also revealed advanced repair.

The cavities located behind the right and left aortic leaflets were similar in appearance. They involved the loose areolar tissue between the roots of the aorta and pulmonic artery and extended into the adventitia of both vessels (fig. 6). The commissure between the right and left pulmonic leaflets and the annulus of the anterior mitral cusp as well as the myocardium of the left atrium, the interventricular septum and the muscle of the conus arteriosus of the mitral ventricle were also involved (fig. 4). The vegetations on the atrial aspect of the anterior mitral cusp marked the points where the cavities approached the surface or had actually perforated. The cavities contained some laminated old blood clot as well as necrotic material, fibrin and degenerating neutrophilic polymorphonuclear leukocytes. The walls consisted of granulation tissue with subacute and chronic inflammatory cell infiltration (fig. 7). Several small and medium sized arteries displayed thrombosis (fig. 8), and revealed marked fibrosis of the adventitia and media without atheromatous changes in the intima.

No microorganisms were identified in any of the lesions. The tricuspid valve and the remaining portions of the pulmonic valve showed nothing of note.

The myocardium revealed hypertrophy of the muscle fibers, some fatty metamorphosis and multiple small areas of old and recent scarring. A few foci of recent acute necrosis of muscle fibers showing infiltration by neutrophilic polymorphonuclear leukocytes were present. A small vessel near a recent scar showed occlusion by amorphous material similar to that composing the valvular vegetations. The pericardium revealed an organizing fibrinous pericarditis. No Aschoff bodies were encountered. The aorta showed some atheromatous plaques but there was no evidence of syphilis.

Additional histologic findings were multiple old and recent infarcts in the lungs, spleen, pancreas, kidneys and cerebellum. There was some chronic passive congestion of all organs with foci of central hemorrhage and necrosis in the liver. The bone marrow showed secondary hyperplasia. The lesion in the right sigmoid was an adenomatous polyp.

Anatomic Diagnosis. Acute bacterial endocarditis, healing, of aortic, mitral and pulmonic valves with mycotic aneurysm of right aortic leaflet; two abscesses of aortic annulus fibrosus with involvement of mitral and pulmonic valve rings, extension into the root of the aorta and pulmonic artery, interventricular septum and left atrium and with rupture into the left and right ventricle and into the sinus of Valsalva of the right aortic and right pulmonic leaflet; fibrosis and calcification of mitral and aortic valves with calcification of mitral and aortic annulus fibrosus; unattached thrombus, left atrium; embolic myocarditis, old and recent; fatty metamorphosis of myocardium; atherosclerosis of coronary arteries; cardiac hypertrophy (550 Gm.); fibrinous pericarditis, healing; multiple infarcts, old and recent, of lung, spleen, pancreas, kidneys and cerebellum; pulmonary edema; chronic passive congestion of viscera; central hemorrhage and necrosis of liver; generalized atherosclerosis, mild; adenomatous polyp of sigmoid; scarred, obliterated appendix; peritoneal and right pleural adhesions, fibrous.

Morphologic Observations

Gross Findings. Endocarditis involved most commonly the aortic valve, while the mitral, tricuspid and pulmonic valves were involved in decreasing frequency (table 1). Involvement of two valves was encountered in 3 instances, involvement of three valves in one.

The endocarditis always showed on at least one valve the typical appearance of acute bacterial endocarditis, modified by antibiotic

therapy (fig. 1*A*). In the case of aortic valve involvement, which was the most common occurrence, the leaflets showed a varying but generally extensive degree of ulceration and perforation with smooth and rounded edges. In many instances, small aneurysmal outpouchings were present. The vegetations were bulky mottled grayish-yellow and -red masses which, on close inspection, revealed endothelialization. The vegetations, however, did not only involve the line of closure of the leaflets proper. They had often spread to the commissures, the fundus of the sinus of Valsalva and to the mural endocardium at or adjacent to the attachment of the valve to the valve ring. (figs. 1*C* and 1*D*). In instances of combined mitral and aortic valve involvement, the mitral vegetation spared the line of closure, but arose near or at the valve base in the region of the valve ring. In the single instance of pulmonic and mitral valve vegetations associated with ulcerative endocarditis of the aortic valve, the pulmonic valve vegetation arose from a commissure and extended into the adjacent sinuses of Valsalva. Vegetations also arose from the mural endocardium of the right ventricle where it joined the involved pulmonic valve commissure (fig. 1*D*).

The atypical location of some vegetations suggested that they had formed secondarily

TABLE 1.—*Tabulation of Principal Findings in 12 Patients with Valve Ring Abscesses Associated with Acute Bacterial Endocarditis*

Patient	Race	Sex	Age	Endocarditis: Valves Involved	Organism	Penicillin Therapy		Pneumonia	Meningitis	Syphilitic Aortitis
						Total Units	Days			
1	W	F	59	AV, MV, PV	Pneumococcus Type 32	9,700,000	12	0	0	0
2	N	M	46	AV, MV	Pneumococcus Type 14	7,100,000	23	+	+	+
3	N	F	62	AV, MV	Pneumococcus Type 15	6,400,000	4	0	+	0
4	N	M	78	AV	Pneumococcus Type 4	4,800,000	15	+	+	+
5	N	M	43	AV	Pneumococcus Type 23	9,600,000	12	0	+	0
6	W	M	64	AV	Pneumococcus Type 9	600,000	7	+	+	+
7	N	M	50	AV	Pneumococcus Type 29	50,000	<1	+	+	+
8	W	M	75	AV	*Staphylococcus aureus*	150,000,000	9	0	0	0
9	N	M	40	AV	*Staphylococcus aureus*	37,700,000	25	0	+	0
10	N	M	36	MV, TV	Pneumococcus Type 4	1,440,000	3	+	0	+
11	N	F	41	MV	Pneumococcus Type 15	4,320,000	27	+	0	+
12	W	F	28	TV	*Staphylococcus aureus*	69,000,000	25	Septic Abortion		0

With aortic valve endocarditis the vegetations sometimes extended to the ventricular aspect of the anterior mitral cusp. Single or multiple crater-like openings ranging from 1 to 5 mm. in size were frequently found. They were located at the junction of mural and valvular endocardium (fig. 1*B*), on the commissures of the semilunar valves, or in the fundus of the sinus of Valsalva. Postmortem blood clots or vegetations often masked these openings. A probe should be inserted into the openings to a depth of a few mm. to 1 to 2 cm. In several instances, the endocarditis involved more than one valve, as occurred in the case reported in detail. Here, the endocarditis of the aortic valve presented the picture as described but vegetations were also present on the mitral and pulmonic valves to an expanding lesion in the underlying tissues. This impression was confirmed by further gross and histologic studies.

The gross recognition of abscesses in the valve rings proved difficult unless certain findings were noted. Upon inspection or palpation, even the largest abscess, which measured 5 cm. in diameter, revealed itself only as a slight and ill-defined swelling. Sometimes a slight bluish discoloration of the overlying endocardium could be noted. The already described crater-like openings (fig. 1*B*) and the atypically located vegetations (figs. 1*C* and 1*D*) were a definite indication of the presence of an abscess. It must be stressed, however, that occasionally abscesses up to 1 cm. in diameter were present which had neither ruptured nor produced vege-

tation and were revealed only when the specimen was sectioned as described.

Upon sectioning, the abscesses were found to range from 3 mm. to 5 cm. in diameter (fig. 2). They tended to follow the course of the valve rings, but in the region of the aortic ring, often spread upward between the root of the aorta and the myocardium of the left atrium. Occasionally they extended for a short distance into the anterior mitral cusp. The outer layers of the aorta and particularly the myocardium of the left atrium were often involved. The lesions were quite well defined and upon sectioning exuded variable amounts of soft reddish-gray or brown material. In many instances, they were partly filled by firm laminated blood clot. The abscesses were multiple in 6 cases. In one case, as many as seven apparently separate lesions were noted. In all but one instance the valve cusp or leaflet adjacent to an abscess showed endocarditis (fig. 9).

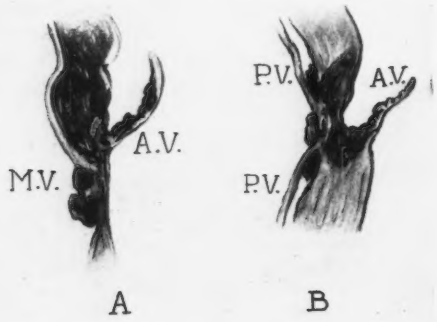


FIG. 2. Cross sections of valve ring abscess associated with acute bacterial endocarditis of aortic valve. (Drawings made from gross photographs.) *A*. Large ring abscess beneath left aortic leaflet extending upwards between root of aorta and left atrium and downwards into mitral ring. Note cross section of vegetation on atrial aspect of anterior mitral cusp (compare with fig. 1*C*.) *B*. Large ring abscess beneath left aortic leaflet extending behind aortic root into commissure between left and right pulmonic valve leaflets. The vegetations arise from this commissure and correspond to those shown in figure 1*D*. Note ulceration of sinus of Valsalva of aortic leaflet.

Histologic Findings. The histologic findings of the valve involved by endocarditis did not differ from the already described picture of this infection when treated by antibiotics (fig. 3).[1,2] In all but 2 instances, the valves showed advanced repair with formation of abundant vascular granulation tissue. The remaining necrotic valve tissue and other necrotic material were partially or completely surrounded and penetrated by granulation tissue. Bacteria could not be demonstrated in the vegetations and endothelialization had occurred. The various stages in the formation of valvular aneurysms as described by Saphir[3] were seen. In 2 patients with staphylococcal infection, the inflammatory process was acute and masses of microorganisms were present. In one of these (case 9) no significant repair could be found while in the other (case 8), healing was slight.

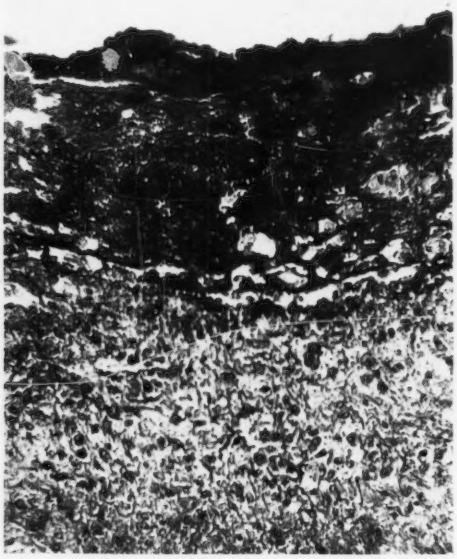


FIG. 3. Healing acute bacterial endocarditis of aortic valve. Granulation tissue is penetrating into the necrotic material of the vegetation. There is no acute inflammatory cell infiltration. Phloxine-methylene blue, × 210.

All abscesses involved at least one of the valve rings. In many instances, the extension of the lesions along the heart skeleton, with involvement of the rings of different valves could be clearly demonstrated (figs. 4, 5 and 6).

The unruptured abscesses showed a core of necrotic material with purulent exudate sur-

rounded by granulation tissue. In some areas the granulation tissue displayed acute inflammatory cell infiltration, while more peripheral areas appeared older, showing organization (fig. 7). In many instances, old laminated blood clot formed part of the contents (fig. 5). Occasional structures resembling microorganisms were seen within polymorphonuclear leukocytes of the acute inflammatory cell exudate. Colonies of microorganisms were not encountered except in two instances of recent staphylococcal infection. The periphery of the lesions showed fibrosis with some chronic inflammation. Hemosiderin deposits were an almost constant finding in the surrounding tissue.

The blood vessels in the abscess walls and surrounding tissues were carefully studied. Occasionally remnants of fragmented and degenerating elastic tissue fibers were found in the abscess wall. In several instances, medium sized arteries near the lesions were occluded, and contained necrotic material similar to that seen in the older portions of the abscesses (fig. 8). Newly formed connective tissue distorted the walls of these vessels, and extended into the thrombus.

The grossly described crater-like openings in the endocardium were seen to represent points of rupture of the abscesses (figs. 4 and 5). The core and the outer layers of the wall of the ruptured lesions were similar to those already described.

The extension of the abscesses occurred by spread of the acute inflammatory process and could often be followed to the surface of one of the heart chambers or to a sinus of Valsalva.

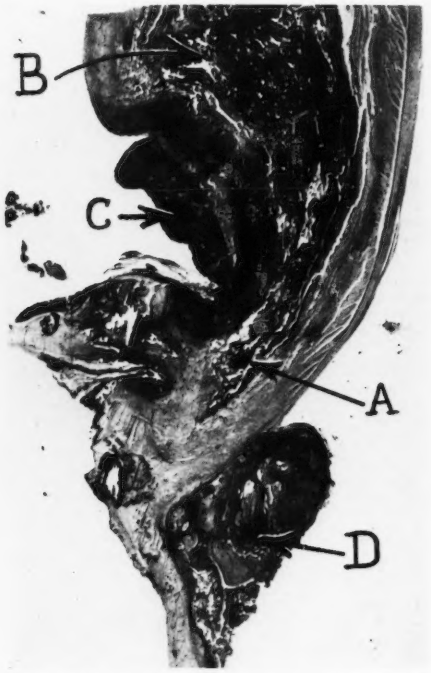


FIG. 4. Abscess of aortic valve ring (*A*) extending upwards between aortic root and wall of the left atrium (*B*). Extension of the abscess to the surface of the sinus of Valsalva has produced ulceration covered by vegetation (*C*). Another vegetation (*D*) overlies the atrial aspect of the mitral ring and on serial sections was shown to have formed secondarily to an extension of the abscess (compare with fig. 2*A*). Phloxine-methylene blue, × 6.5.

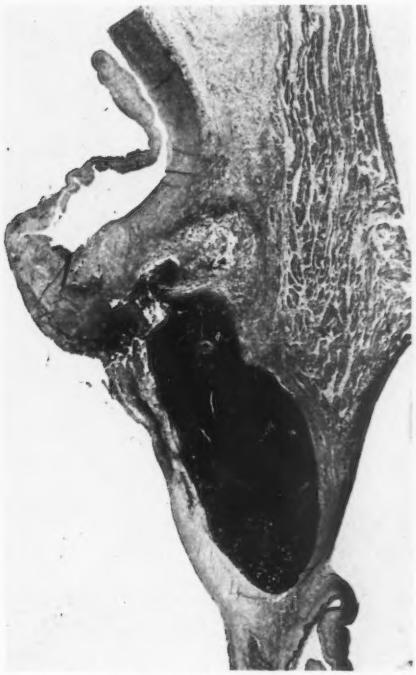


FIG. 5. Abscess involving both aortic and mitral rings partly filled with laminated thrombus. The abscess has ruptured through mural endocardium beneath aortic leaflet (left center). Phloxine-methylene blue, × 6.5.

Vegetations formed when the underlying inflammatory process approached the endothelial surface (fig. 4). Various stages in the formation of vegetation could be observed from minute histologic thrombi to bulky masses, but their degree of repair was similar to that noted in the primarily infected valves.

FIG. 6. Extension of aortic valve ring abscess (*A*) to pulmonic ring. The abscess has ruptured into the sinus of Valsalva (*B*) of the aortic leaflet (*C*). Vegetations (*D*) cover the endothelium on the pulmonic side where the abscess approaches the surface. Phloxine-methylene blue, × 6.5.

Other Findings. The heart weights varied from 240 to 790 Gm., with an average of 498 Gm.

The valves not involved by endocarditis appeared grossly and histologically normal. Calcification of the aortic valve annulus, with some involvement of the leaflets, was present in 2 cases. Calcification of the mitral valve annulus was noted in 3 instances. Embolic myocarditis was an almost constant finding, and showed advanced healing. In one patient with staphylococcal endocarditis (case 12) a 3 mm. abscess was present in the tip of the right papillary muscle of the left ventricle. Morphologic evidence of syphilitic aortitis was found in 6 patients (table 1).

A wide variety of peripheral embolic complications was encountered, which did not differ from those commonly found in this disease. In most instances, these showed advanced healing. In 2 patients with staphylococcal endocarditis, embolic brain abscesses appeared to

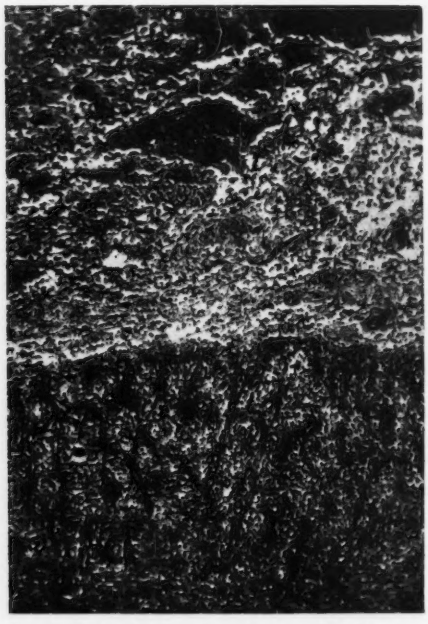


FIG. 7. Wall of valve ring abscess with the necrotic and purulent contents (top) surrounded by granulation tissue with acute inflammatory cell infiltration (bottom). Phloxine-methylene blue, × 96.

be of clinical significance and in one the brain showed multiple areas of infarction. A fortunate cut revealed that an area of infarction was associated with a classic mycotic aneurysm of a cerebral vessel.

In all patients but one who entered the hospital with pneumonia, meningitis or both, these infections showed advanced or complete healing at autopsy.

CLINICAL FINDINGS

Some of the clinical data are summarized in table 1.

Our patients varied in age from 28 to 78 years. There were 8 men and 4 women.

The duration of acute illness before hospitalization varied from one day to five weeks but in most instances was about three weeks. All patients were acutely ill when admitted, and usually a history had to be obtained from the family. All had had an acute febrile illness, and classic symptoms of pneumonia or meningitis were elicited in several instances. The past history of one patient revealed lobar pneumonia six months prior to his present illness. He had received penicillin therapy at that time. Another patient had had a criminal abortion eight days before admission.

The past history of 7 patients indicated organic heart disease, and 6 of these had experienced symptoms of congestive heart failure. In 3 of the 7 patients syphilitic aortic insufficiency had been previously diagnosed, and syphilitic aortitis was found at autopsy in 2 others. The history of a further patient suggested longstanding rheumatic heart disease.

Seven patients had previously received antisyphilitic therapy or had positive serologic tests for syphilis at the time of admission. At autopsy 6 of these were found to have morphologic evidence of syphilitic aortitis. The seventh gave a history of antisyphilitic therapy and had a strongly positive serologic test, but no evidence of the disease could be found at postmortem examination.

Eight patients were irrational or comatose when first seen in the hospital. Six had clinical and laboratory findings of pneumonia. A diagnosis of meningitis was established in 6 patients, and an additional patient was found to have meningitis at autopsy. Both pneumonia and meningitis were present in 4 patients.

All patients received penicillin therapy in the hospital. One died shortly following an initial administration of 50,000 units. The other patients received total doses ranging from 600,000 to 150 million units over periods from 3 to 27 days. In addition, sulfadiazine was given to 6 patients, aureomycin to 2 and streptomycin to 2. No reliable information is available concerning therapy before admission. A subsiding meningitis and pneumonia encountered in the patient dying within three hours after admission suggest that he had received previous antibiotic therapy.

Nine of the 12 patients remained febrile up to the time of death. Three of the 9 patients showed no clinical evidence of congestive heart failure, 4 had minimal findings and 2 were in moderately severe failure. Two of the 12 became afebrile, one on the fourth, the other on the sixth day of therapy. Both, however, died of severe progressive heart failure. The last patient died with a massive encephalomalacia, possibly embolic in origin.

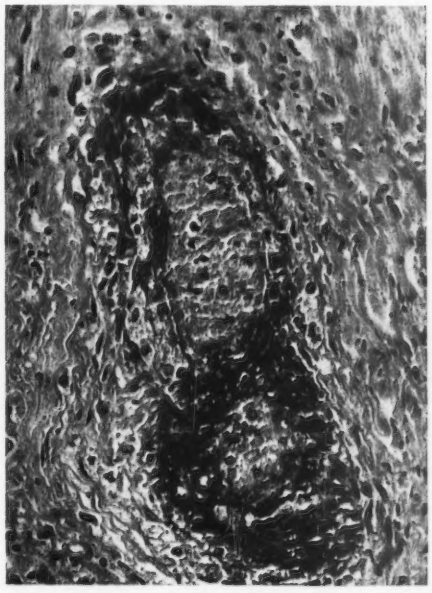


FIG. 8. Occluded blood vessel at periphery of valve ring abscess. Phloxine-methylene blue, × 400.

BACTERIOLOGIC FINDINGS

The causative organism in 9 patients was pneumococcus and in 3 *Staphylococcus aureus* (coagulase positive in 2, coagulase negative in one) (table 1). Multiple blood cultures were positive in all patients on admission and in 6 the organism was also recovered from the spinal fluid. Blood cultures were repeated during treatment in 5 patients. The cultures of 3 patients with pneumococcal infection yielded no growth. The cultures of 2 patients with staphylococcal endocarditis continued to be positive.

During the early phases of this study we did

not appreciate the importance of thorough postmortem bacteriologic study, and in most instances failed to obtain cultures from vegetations and valve ring abscesses. Cultures of vegetations were obtained from 6 patients. Pneumococcus was recovered from two, *Staphylococcus aureus* from three and one vegetation proved sterile. Valve ring abscesses were cultured in only 3 cases and all were positive. *Staphylococcus aureus* was grown from two and pneumococcus from the third. Cultures of heart blood and lung were taken at autopsy in all 12 cases. A positive blood culture was obtained in only one patient with pneumococcal endocarditis and in no instance was the pneumococcus recovered from lung. *Staphylococcus aureus* was grown from heart blood or lung in all 3 patients with this infection.

Discussion

Abscesses in the valve rings occurred in 12 of 14 cases of acute bacterial endocarditis which came to autopsy between 1947 and 1950. Similar lesions, although searched for, were not encountered in 11 cases of subacute bacterial endocarditis. The abscesses were often of appreciable size, but inconspicuous on routine examination of the heart. The presence of the lesions could be suspected from certain gross findings. Sometimes a swelling was noted in the region of the valve rings, occasionally associated with a bluish discoloration of the endocardium. Crater-like openings in the mural endocardium near the valve rings and in the sinuses of Valsalva, as well as on the commissures of the semilunar valves, were frequently encountered and found to represent points of rupture of underlying abscesses. The most striking finding was vegetations which arose near or at the valve ring. The free edge and line of closure of these valves were uninvolved. These atypically located vegetations had formed secondarily to the extension of the underlying abscess. In all but one instance the abscesses involved the ring of a valve cusp or leaflet which showed the typical picture of primary involvement by acute bacterial endocarditis. Here the vegetations always involved the free edge and line of closure of the valve. The abscesses were often multiple and sometimes extended from one valve ring to another (fig. 9).

On histologic examination, the abscesses consisted of a necrotic center surrounded by granulation tissue. Old laminated thrombi were often part of the core. All abscesses showed areas of acute and progressive inflammation, as well as repair. In some instances the tissue surrounding the abscesses revealed arteries occluded by organizing thrombi.

In all but 2 patients (cases 8 and 9) the valves, the vegetations, and the embolic lesions in the myocardium as well as in other organs, showed subsidence of the acute inflammatory process and advanced repair. The valve ring abscesses appeared to be the only obvious foci of acute inflammation.

We were surprised at the frequency of valve ring abscesses in our cases of acute bacterial endocarditis. These lesions may have been overlooked by us in the past, or may represent a new aspect of acute bacterial endocarditis developing in association with more effective therapy. The incidence of these lesions cannot be determined from a review of the recent literature since they have not attracted attention. Careful study of autopsy protocols, however, included in reports on acute bacterial endocarditis revealed that similar lesions have occasionally been encountered.[4-10] Of the standard textbooks of pathology, Karsner[11] and Aschoff[12] mention only the rare occurrence of abscesses in the myocardium following the extension of acute bacterial endocarditis. MacCallum[13] describes cavities filled with infected thrombus in the sinuses of Valsalva which he called mycotic aneurysms. He noted perforations as well as extension into the walls of the atria and the interventricular septum. Ribbert[14] describes the lesions in detail as a rare occurrence in acute bacterial endocarditis and states that they developed from a spread of the infection from the valves to the sinuses of Valsalva and the aortic root. His text figure 18 illustrates well their location and general histologic appearance. Except for these observations, the morphology and pathogenesis of the abscesses have apparently not been investigated. We

have found no references to their possible clinical significance.

The pathogenesis of the valve ring abscesses remains uncertain, since despite extensive study, we have never encountered the early stage of the lesions. Their histologic appearance, with laminated thrombi in the center and occluded blood vessels in the adjacent tissue, suggests that they are mycotic aneurysms of the vessels in the valve ring. The peripheral vessels in acute bacterial endocarditis frequently show similar aneurysms which are the result of septic emboli.[15]

If the valve ring lesions are mycotic aneurysms, increased vascularity of the valve ring, subsequent to syphilitic aortitis, rheumatic heart disease, or to atherosclerosis could present a predisposing factor. It might therefore be significant that 6 of our patients had syphilitic aortitis and that 3 patients showed calcification of the mitral ring.

The anatomic relationships of the four valve rings explain the involvement of more than one of these structures by abscesses (fig. 9). The aortic and mitral valves share a common ring in part of their circumference, and the rings of the tricuspid and pulmonic valves are in close apposition to segments of the aortic ring.

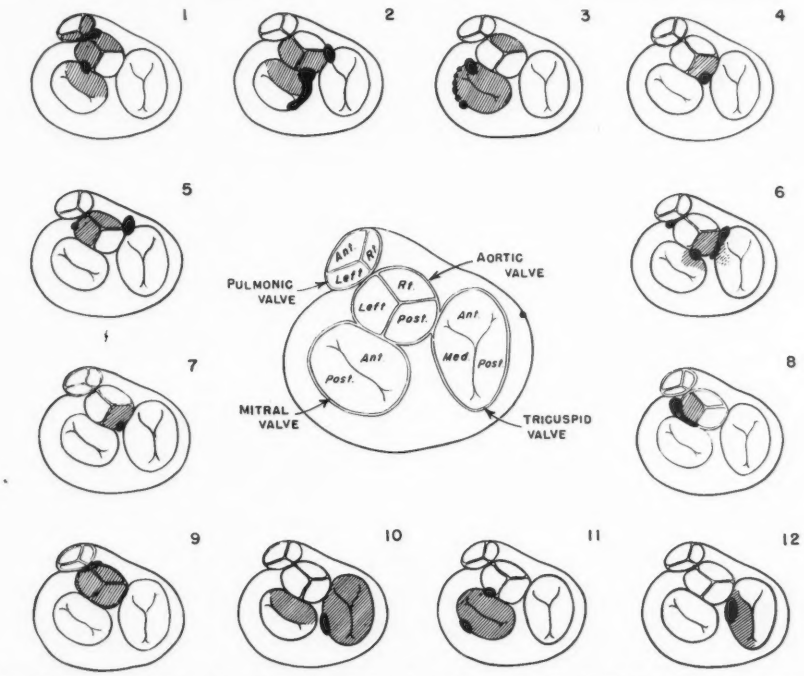


FIG. 9. The drawing in the center shows the close anatomic relationships of the four valve rings as viewed from above after removal of the atria and the great vessels. The satellite drawings indicate the number and location of the valve ring abscesses (solid black) as well as the valves involved by endocarditis (shaded).

Our study revealed no criteria for the clinical recognition of the valve ring abscesses. We feel, however, that the lesions may be of clinical significance. Several of our patients died with clinical evidence of persistent infection but without significant congestive heart failure. At autopsy the valvular and other lesions such as pneumonia and meningitis showed generally advanced healing while the valve ring abscesses

appeared to represent the only site of active inflammation. Despite vigorous administration of antibiotics, the therapeutic results in acute bacterial endocarditis have been unsatisfactory. This failure may in part be related to abscesses of the valve rings.

It is our impression that the valve ring lesions are actually more frequent now than in the past. Prior to modern therapy, most patients died early in the course of acute bacterial endocarditis with unchecked destruction of the valves and massive bacteremia. Antibiotics appear to control the infection on the valves as well as in other primary or metastatic sites, but may not penetrate the valve ring in therapeutic concentrations. The same factors which prior to antibiotic therapy prevented the healing of the valvular lesions may still obtain in the valve rings and account for the progression of infection in the valve ring abscesses.

Summary

Single or multiple abscesses of the valve rings of the heart were encountered at autopsy in 12 patients with acute bacterial endocarditis who had received penicillin therapy. The abscesses measured from 1 to several centimeters in size and, because of the close anatomic relationships of the valve rings, frequently involved more than one of these structures. Rupture of the lesions either into the chambers of the heart or into the sinuses of Valsalva of the semilunar valves occurred in many instances.

The abscesses appeared to originate from mycotic aneurysms of vessels in the valve rings. Increased vascularity of these structures subsequent to syphilitic aortitis, rheumatic heart disease or atherosclerosis might represent a predisposing factor.

The valve ring abscesses were the only recognizable foci of persistent acute inflammation while the endocarditis and its peripheral embolic lesions as well as the associated pneumonia and meningitis generally showed advanced healing.

It is our impression that abscesses in the valve rings are encountered more frequently now than in the past. The unsatisfactory therapeutic results in acute bacterial endocarditis may be related to these lesions. Antibiotic agents control the infection on the valves as well as in the peripheral embolic and other associated lesions, but do not appear to penetrate the valve ring abscesses in sufficient concentration to prevent their progression.

References

[1] Geiger, A. J., and Durlacher, S. H.: The fate of endocardial vegetations following penicillin treatment of bacterial endocarditis. Am. J. Path. **23:** 1023, 1947.

[2] Moore, R. A.: The cellular mechanism of recovery after treatment with penicillin. I. Subacute bacterial endocarditis. Tr. & Stud. Coll. Physicians, Philadelphia. **14:** 55, 1946.

[3] Saphir, O., and Leroy, E. P.: True aneurysms of the mitral valve in subacute bacterial endocarditis. Am. J. Path. **24:** 83, 1948.

[4] Claytor, T. A.: Spontaneous rupture of the heart in a case of ulcerative endocarditis. J. A. M. A. **80:** 1371, 1923.

[5] Weiss, S., and Wilkins, R. W.: Myocardial abscess with perforation of the heart. Am. J. M. Sc. **194:** 199, 1937.

[6] Bain, C. W. C., and Wray, S.: Ruptured aortic valve with mycotic aneurysm due to acute bacterial endocarditis. Brit. Heart J. **3:** 132, 1941.

[7] Bartol, G. M., Edwards, J. E., and Lamb, M. E.: Mycotic and dissecting aneurysms of the aorta complicating bacterial endocarditis. Arch. Path. **35:** 285, 1943.

[8] Pirani, C. L.: Erosive (mycotic) aneurysm of the heart with rupture. Arch. Path. **36:** 479, 1943.

[9] Vilter, C. F., and Ritterhoff, R. J.: Mycotic aneurysm of the sinus of Valsalva. Ohio State M. J. **41:** 246, 1945.

[10] Close, W. D., McKinley, A. D., and Michael, A. C.: Mycotic aneurysm of sinus of Valsalva in bacterial endocarditis while under treatment with penicillin. Quart. Bull. Indiana Univ. M. Center. **10:** 3, 1948.

[11] Karsner, H. T.: Human Pathology, ed. 7. Philadelphia, J. B. Lippincott, 1948. P. 394.

[12] Aschoff, L.: Pathologische Anatomie, ed. 7. Jena, Gustav Fischer, 1928. Vol. 2, p. 28.

[13] MacCallum, W. G.: A Textbook of Pathology. ed. 7. Philadelphia, W. B. Saunders, 1940. P. 248.

[14] Ribbert, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie. Berlin, Julius Springer, 1924. Vol. 2, Herz und Gefässe, p. 226.

[15] Forbus, W. D.: Reaction to Injury, Baltimore, Williams & Wilkins, 1943. P. 432.

The Use of Procaine Amide in Cardiac Arrhythmias

By HERBERT J. KAYDEN, M.D., J. MURRAY STEELE, M.D., LESTER C. MARK, M.D., AND BERNARD B. BRODIE, PH.D.

Procaine amide, a new synthetic analogue of procaine, has marked antiarrhythmic activity and is relatively stable in the body. It is effective when administered orally or intravenously, and has few toxic effects in therapeutic doses. The drug has been used successfully in the suppression of ventricular premature contractions and in the interruption of ventricular tachycardia. Some patients treated successfully with procaine amide had been given quinidine to the point of toxicity without affecting the aberrant rhythm. Procaine amide appears to be less effective in interrupting auricular than ventricular arrhythmias.

PROCAINE, administered intravenously, exerts an antiarrhythmic action on the heart.[1] However, its stimulatory effects on the central nervous system limit its application as an antiarrhythmic drug to the anesthetized patient. In addition, procaine is rapidly inactivated by its hydrolysis in the blood stream to p-aminobenzoic acid and diethylaminoethanol. The latter compound in rather large dosage also exhibits antiarrhythmic action, but with minimal stimulatory effects on the central nervous system. In a previous communication, experiences with diethylaminoethanol and its possible value in ventricular tachycardia were discussed.[4]

A series of derivatives of diethylaminoethanol were synthesized* in the hope that the activity of the alcohol could be enhanced without a comparable increase in toxicity and that a compound would be found which would have greater stability than procaine. The compounds were tested for antiarrhythmic activity on the ventricular tachycardia which is induced by injection of epinephrine into dogs anesthetized with cyclopropane. The most promising compound of the series was procaine amide,† the amide analogue of procaine. This report is concerned with results obtained in treating various cardiac arrhythmias with procaine amide.

The pharmacologic data on procaine amide has been detailed elsewhere.[3] Briefly, the drug is rapidly and completely absorbed from the gastrointestinal tract, peak blood concentrations being obtained one to two hours after its oral administration. It is relatively stable in the body, not being affected by the enzyme which catalyzes the hydrolysis of procaine. It is slowly excreted by the kidneys, plasma levels declining only about 15 per cent per hour. About 60 per cent of the drug is excreted unchanged in the urine. In patients with normal renal function, plasma levels reach a relative plateau after 36 to 48 hours of repeated oral administration of a constant amount.

The most common arrhythmia available for study was the premature contraction of ventricular origin. The effects of procaine amide were observed in 54 patients with this condition. In some patients, the premature beats were attributable to the administration of digitalis, while in others they were associated with organic, primarily arteriosclerotic heart disease.

From the Third (New York University) Medical and Research Services, Goldwater Memorial Hospital, Welfare Island, N. Y., and the Department of Anesthesiology, New York University-Bellevue Medical Center, New York, N. Y., and the National Heart Institute, National Institutes of Health, Bethesda, Md.

A preliminary report of this study was presented at the annual meeting of the American Heart Association, June, 1950.

During the course of this work, one of us (L. C. M.) was a Research Fellow of the American Heart Association.

* By W. A. Lott, E. R. Squibb & Co., New Brunswick, N. J.

† Supplied as Pronestyl hydrochloride by E. R. Squibb & Co.

In each instance, the drug administered orally or intravenously in doses varying from 0.4 to 1.0 Gm., suppressed the ectopic beats. On intravenous administration the effect occurred during or shortly following the period of injection; after oral administration there was usually a delay of 30 to 60 minutes. After a single intravenous dose, the period of suppression usually varied from three to six hours. The irregularity of the occurrence of extrasystoles makes it difficult, however, to estimate the duration of effect of the drug. In an occasional patient suppression has lasted for less than one hour, and in a few for more than 24 hours. Experience with oral administration has helped in estimating the duration of effect. Following a single oral dose, the usual period of suppression appeared to be the same as with an intravenous dose, namely three to six hours. In 14 cases, the administration of procaine amide at intervals of three to six hours successfully prevented the recurrence of ventricular extrasystoles for many weeks. In one patient, therapy has been continued successfully for four months.

The effect of procaine amide on ventricular tachycardia was studied in 15 patients with this disturbance. In 13 of these, administration of the drug orally, intravenously, or by both routes was successful in that there was reversion to the cardiac rhythm that existed prior to the ventricular tachycardia. (Three examples are shown in figures 1, 2 and 3.) There were two failures. One patient (case 3), who presented an unusually difficult problem, died during intravenous injection of procaine amide. In retrospect, too rapid administration of the drug may have been responsible for the development of ventricular fibrillation. In the other patient (case 9), death due to ventricular fibrillation occurred during administration of procaine amide, but before a significant quantity (150 mg.) had been administered. A resume of the clinical findings and therapy for each of the treated cases is appended.

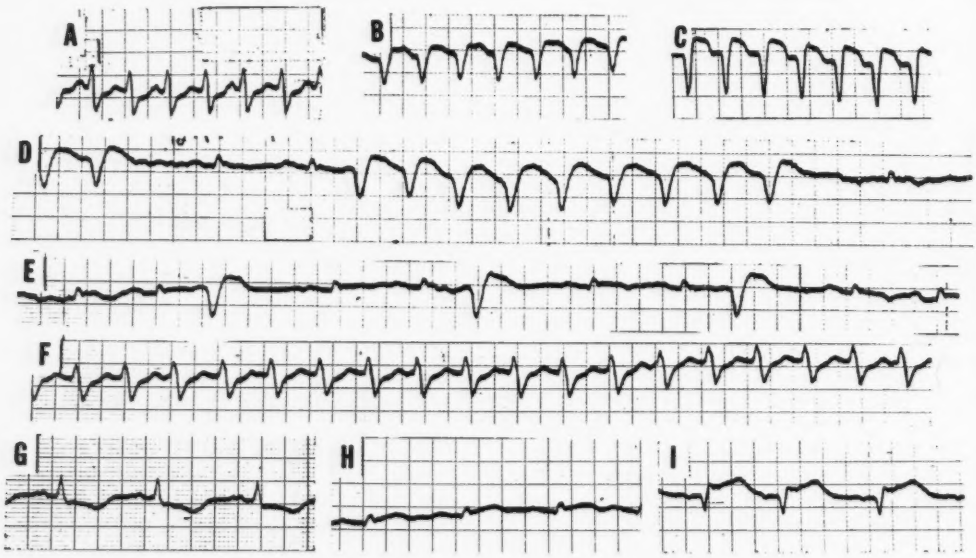


FIG. 1. Effect of procaine amide on ventricular tachycardia (case 5). *A*, lead I; *B*, lead II, and *C*, lead III: control tracings. Ventricular tachycardia. Rate 190 per minute. *D*. After injection 600 mg. procaine amide (lead II). *E*. After injection 1 Gm. procaine amide (lead II). Maintenance oral therapy 1 Gm. every three hours (see case report). *F*. Four hours later (lead I). Ventricular tachycardia recurred. Dosage increased to 1 Gm. every two hours. *G*, lead I; *H*, lead II; and *I*, lead III: 12 hours later. Normal sinus rhythm. Rate 78.

Analysis of the electrocardiograms obtained

during intravenous injections indicates that the sequence of events incidental to the interruption of ventricular tachycardia may vary considerably. In some cases, the ventricular tachycardia is abruptly interrupted and a supraventricular pacemaker, frequently the A-V nodle, is established. This occurs with or without preliminary slowing of the ventricular rate. In other patients, the aberrant rhythm does not terminate abruptly; occasional beats of supraventricular origin first interrupt the ventricular tachycardia, increase in frequency during a period of several minutes, until the supraventricular pacemaker is established. The course of response is not predictable.

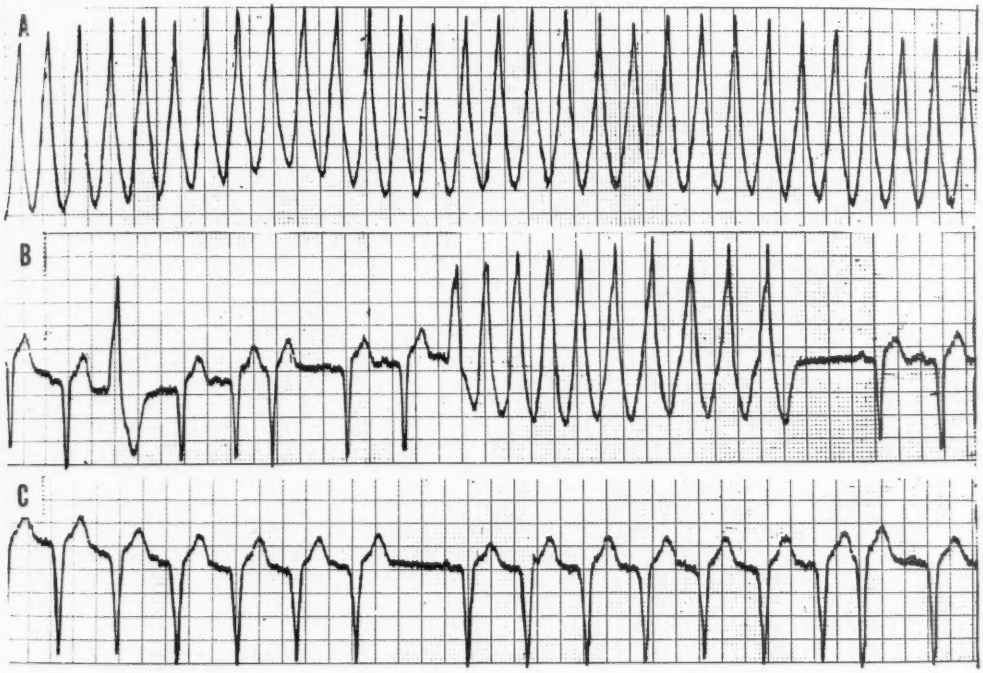


FIG. 2. Effect of procaine amide on ventricular tachycardia (case 6) (lead III, retouched). *A*. Control tracing. Ventricular tachycardia. Rate 194 per minute. *B*. After injection 200 mg. procaine amide. *C*. After injection 400 mg. procaine amide.

The treatment of patients with auricular arrhythmias was much less successful. Auricular premature contractions could be eliminated by intravenous administration of procaine amide, but the effect was of shorter duration than with ventricular premature contractions. Apparently the duration of the abnormal rhythm is of considerable importance. Ten patients with chronic auricular flutter and 14 patients with chronic auricular fibrillation were treated with 1 Gm. of procaine amide injected intravenously over a period of five minutes. In none of these patients was normal sinus rhythm reestablished, but an effect on auricular activity was observed in that there was marked slowing of the *f* waves (fig. 4). In 2 patients with recently established auricular fibrillation, however, normal rhythm appeared during the intravenous administration of 1 Gm. of procaine amide and persisted for several days. Further exploration of the effect of the drug on auricular fibrillation of recent origin seems warranted.

Two patients with nodal tachycardia were treated with procaine amide. One patient reverted to normal rhythm once on oral administration (3 Gm. in divided doses) and twice on

intravenous injection of 1 Gm. (fig. 5). The other patient did not respond to 1 Gm. given intravenously. This patient was not treated further with the drug.

The mechanism of action of procaine amide is not completely understood. The refractory period, as measured by Q-T interval, is prolonged and conduction is apparently slowed. In animal studies, procaine amide has been shown to increase the threshold to electrical stimulation of the ventricle.[2]

patients receiving a single dose, but may persist in patients on oral maintenance therapy. The development of ectopic beats as a result of the injection of procaine amide has not been observed. Administration of procaine amide to patients who already had signs of digitalis or quinidine toxicity did not result in untoward reactions.

Toxic effects have been limited to the circulatory and gastrointestinal systems. Anorexia, nausea, and vomiting were present only on high

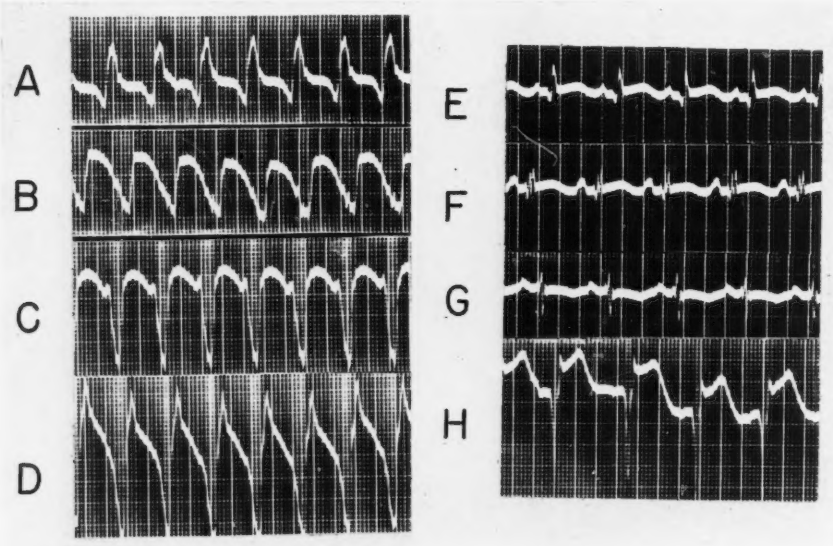


FIG. 3. Effect of procaine amide on ventricular tachycardia (case 8). *A*, lead I; *B*, lead II; *C*, lead III; and *D*, lead CF_4; Control tracings. Ventricular tachycardia. Rate 148 per minute. *E*, lead I; *F*, lead II; *G*, lead III; and *H*, lead CF_4: Four hours after injection 1 Gm. procaine amide. Normal sinus rhythm. Rate 94.

Electrocardiographic changes were noted in approximately one-third of patients receiving procaine amide orally or intravenously.* They have consisted in widening of QRS and Q-T intervals and diminution of voltage of QRS and T waves. The changes are transient in

* In animal experiments, the continuous intravenous infusion of procaine amide to fatality produced the following cardiac disturbances: prolongation of P-R interval, widening of the QRS complexes, wandering pacemaker, nodal and ventricular premature contractions, ventricular tachycardia and fibrillation.

oral therapeutic schedules (6 to 8 Gm. per day). Following the intravenous administration of procaine amide, transient hypotension has occurred in about one-third of patients who did not have ventricular tachycardia. In the group with ventricular tachycardia, in whom hypotension is already frequently present, intravenous administration of procaine amide has usually produced a more marked depression, but this promptly disappeared with the establishment of a supraventricular rhythm. Hypotensive effects have not been

observed with oral administration even in the nine bouts of ventricular tachycardia successfully treated by this route.

Any dosage schedule based on the limited number of cases so far studied is, naturally, a provisional one. The intravenous route is preferable only in patients who are critically ill or who are unable to take oral medication. The maximal rate of intravenous administration has been 200 mg. per minute until aberrant rhythm has been interrupted or until a total of 1 Gm. has been administered. It is helpful to have an electrocardiogram, preferably recorded with a direct-writing instrument, during the injection. Because blood pressure often falls, it also should be frequently recorded. When the drug is given orally to patients, 1.25 Gm. may be given initially, and if the electrocardiogram reveals no change in one hour, a second dose of 0.75 Gm. Further doses of 0.5 to 1 Gm. may be administered every two hours as required until the aberrant rhythm is eliminated. Maintenance oral therapy may be necessary to prevent recurrence of extrasystoles and paroxysms of ventricular tachycardia. Doses of 0.5 to 1 Gm. administered every three to six hours during the day and night have maintained normal rhythm in patients studied to date. Similar oral schedules are recommended for ventricular premature contractions—requirements have varied from 1.5 to 8 Gm. per day in divided doses. Since the drug is excreted to a large extent by the kidneys, patients with impaired renal function receiving oral maintenance therapy may achieve unusually high plasma levels.

Further investigation is needed to evaluate the place of procaine amide in the treatment of cardiac arrhythmias. It would appear to be a safer and more effective agent than quinidine in the management of ventricular tachycardia and ventricular extrasystoles. Its application to the control of arrhythmias in anesthesia and in cardiac surgery is at present under study. Initial reports indicate that the prophylactic administration of procaine amide materially diminishes the incidence of arrhythmias during intrathoracic surgery.[5]

Case Reports

Case 1. W. W., a 77 year old male, was admitted in 1947 with acute urinary retention. He had been on maintenance digitalis therapy for congestive heart failure for several years following a myocardial infarction. There was no history of hypertension. In November 1948 he complained of palpitations and an electrocardiogram revealed a supraventricular tachycardia at a rate of 150 per minute. Oral quinidine sulfate and additional digitalis medication slowed the rate to 80, but the rhythm remained that of auricular flutter with a varying block. Seven months later, the patient had sudden precordial pain, dyspnea and palpitation. An electrocardiogram revealed ventricular tachycardia. Procaine amide was given intravenously four hours after the onset; at the end of five minutes (432 mg.), the ventricular tachycardia ceased and the rhythm of auricular flutter was renewed. Thirty-five minutes later the aberrant ventricular rhythm reappeared, and persisted for 25 minutes. The additional injection of 432 mg. over a five minute period again established

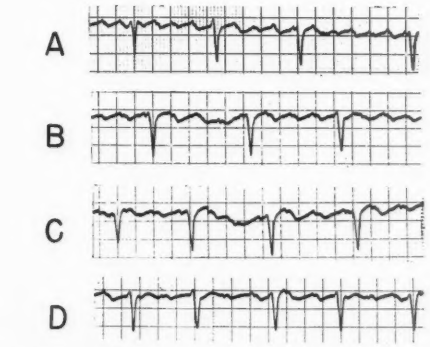


Fig. 4. Effect of procaine amide on auricular flutter (right parasternal lead). *A*. Control tracing. Auricular rate 300 per minute. Ventricular rate 62. Ventricular premature contractions. *B*. After injection of 600 mg. procaine amide. Auricular rate 240. Ventricular rate 66. *C*. After injection 1 Gm. procaine amide. Auricular rate 196. Ventricular rate 82. *D*. Five minutes later. Auricular rate 187. Ventricular rate 86.

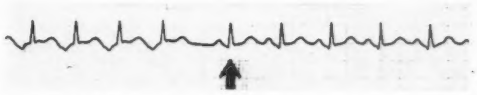


Fig. 5. The effect of procaine amide on nodal tachycardia. (Lead II retouched). Rate before injection 150 per minute; on completion of injection of 850 mg. (arrow), regular sinus tachycardia 110.

auricular flutter. Subsequent clinical and electrocardiographic findings confirmed the impression of a new myocardial infarction. The patient had an uneventful convalescence, although his rhythm remained that of auricular flutter. Two months later he fell and died within 24 hours of a fractured skull. Postmortem examination was not obtained.

Case 2. M. G., a 60 year old white male, was admitted to the hospital with a diagnosis of acute myocardial infarction. On the fifth hospital day, he developed ventricular tachycardia. During the next 48 hours he was treated with quinidine sulfate orally to toxicity and magnesium sulfate intravenously, but there was no change in the rhythm. Then 0.5 Gm. of procaine amide was injected. At the end of injection (five minutes), occasional beats of sinus origin appeared, but the dominant rhythm remained that of ventricular tachycardia. Twenty minutes later, 0.5 Gm. of procaine amide was again given intravenously, and resulted in the appearance of more beats of sinus origin, which, however, did not persist for long. In the succeeding 48 hours the intravenous injection of quinidine sulfate, atabrine and diethylaminoethanol failed to interrupt the ventricular tachycardia. On the fifth day of the sustained ventricular tachycardia, procaine amide was again tried intravenously. One Gm. given over a 10 minute period had no demonstrable effect; 35 minutes later a second dose of 1 Gm. administered over a five minute period produced frequent sinus beats that established trigeminy. An additional dose of 0.25 Gm. intravenously was given 20 minutes later. The ventricular premature contractions disappeared completely in 40 minutes and the patient had an entirely uneventful hospital convalescence. He was hospitalized again 18 months later because of congestive heart failure. He responded promptly to treatment and was discharged two weeks after admission.

Case 3. S. L., a 45 year old male, was admitted to the hospital with a history of sudden chest pain and palpitation. Two years previously he had sustained a myocardial infarction. During the year prior to the second admission, he had observed increasing angina on effort. On admission, an electrocardiogram revealed ventricular tachycardia. Oral and intravenous therapy with quinidine administered to toxicity, as well as intravenous magnesium sulfate, did not alter the rhythm. On the third hospital day, 1 Gm. procaine amide administered intravenously in five minutes had no effect. An additional Gm. 30 minutes later produced occasional sinus beats (one per six to seven ventricular beats) for about 25 minutes. A third dose of 1 Gm. in three and a half minutes had no effect. That night 0.6 Gm. quinidine lactate was given intravenously, without producing either toxic or therapeutic effect. The next day the patient appeared moribund. An attempt was made to give 2 Gm. procaine amide at a faster rate, that is 400 mg. per minute. After the injection of 1 Gm. in two and a half minutes (one half contemplated dose), the patient developed ventricular fibrillation and died. At autopsy extensive sclerosis of the coronary arteries was found. There was complete occlusion of left coronary artery and a recent thrombus in right coronary artery with fresh necrosis in the papillary muscle of left ventricle. The entire wall of the left ventricle was thin and fibrotic, and at the apex there was an area of calcification.

Case 4. W. M., a 52 year old white male office worker, was admitted to the hospital following collapse on the street. He had been admitted three years before with a diagnosis of myocardial infarction. During the year prior to second admission he had noted polyuria, and had lost 60 pounds. During the weeks just prior to admission, the patient complained of anorexia with nausea and vomiting, polydypsia and increasing retrosternal pain on moderate effort. Examination on admission showed an acutely ill, pallid, apprehensive male; his blood pressure was 100/80 and his apical rate was 144 per minute. Examination of the urine showed a specific gravity of 1.032, 4 plus glycosuria and 2 plus acetonuria. After 12 hours of vigorous treatment with insulin and appropriate fluids, the urine became free of glucose and ketone bodies. During this period he was also digitalized with 1.2 mg. of digitoxin because of signs of congestive heart failure. An electrocardiogram taken at the end of this period of therapy revealed ventricular tachycardia. Oral quinidine was administered to toxicity. He was able to tolerate 3 Gm. daily in divided doses for the first five days, and then only 1.5 Gm. daily in divided doses for second five days. No effect on the rhythm was noted, though the QRS interval widened considerably. On the eleventh hospital day procaine amide was given intravenously; at the end of three and a half minutes (642 mg.), the aberrant rhythm was abruptly terminated, and normal sinus rhythm reestablished. The patient had an entirely uneventful convalescence and was discharged at the end of six weeks.

Case 5. E. F., a 65 year old white woman, was known to have had hypertension and diabetes mellitus for the prior 10 years. She was admitted to the hospital with a history of increasing dyspnea on effort and nocturnal orthopnea for two weeks and severe right upper quadrant pain for 24 hours. Physical examination showed an obese cyanotic female, in marked respiratory distress. Pulse rate was 90; blood pressure, 160/90. Examination of the lungs revealed many moist rales bilaterally; there was moderate distention of veins in the neck and a large tender liver extending 4 fingerbreadths below costal margin. Venous pressure was 200 mm. citrate and Decholin circulation time was 35 seconds. An electrocardiogram showed a rate of 90 per minute and changes consistent with posterior wall myocardial infarction. Oxygen, anticoagulants, penicillin

and digitalis (0.8 mg. digitoxin by mouth in six hours) were administered. The following morning the patient had developed a rapid cardiac rate of 200 per minute, which was shown by electrocardiogram to be ventricular tachycardia (fig. 1*A*, *B*, *C*). The attempted oral administration of 0.9 Gm. of quinidine was unsuccessful because of vomiting. Quinidine lactate, 0.65 Gm., was given intravenously in 250 cc. solution over a period of 75 minutes, and resulted in slowing of the rate from 170 to 140. Three and a half hours later, the administration of 1.4 Gm. procaine amide intravenously in divided doses over 30 minutes, established bigeminal and then trigeminal rhythm (fig. 1*D*, *E*). Oral therapy of 1 Gm. every three hours was started, but after the second dose, ventricular tachycardia recurred (fig. 1*F*). The dosage schedule was increased to 1 Gm. every two hours, and 12 hours later an electrocardiogram showed normal sinus rhythm at a rate of 72 (fig. 1*G*, *H*, *I*). Dosage was then reduced to 1 Gm. every three hours and the patient maintained normal sinus rhythm. The blood pressure which had been about 90/60 during her tachycardia, rose to 130/80. Twelve hours later the patient suddenly became very dyspneic and expired almost immediately. Permission for postmortem examination was refused.

Case 6. C.O., a 70 year old white female, was known to have had hypertension for 20 years. She had been bedridden for the past 10 years following a left hemiplegia. There was no history of cardiac decompensation. Electrocardiograms obtained during these 10 years of hospitalization had revealed normal sinus rhythm with wandering pacemaker and occasional ventricular premature contractions.

Suddenly, on Jan. 9, 1950, she noticed severe palpitation and an electrocardiogram showed ventricular tachycardia at a rate of 200 per minute. Procaine amide was given intravenously; after 200 mg. had been injected, beats of supraventricular origin appeared. At the end of the injection of 1 Gm., the ectopic ventricular beats had disappeared and the pacemaker appeared to be located in the auriculoventricular node. The electrocardiogram at the termination of injection, showed changes suggestive of fresh myocardial injury, but during the next four hours these abnormalities disappeared and normal sinus rhythm was established. An electrocardiogram on the next day (1/10/50) showed occasional ventricular premature contractions and bursts of ventricular tachycardia. The oral administration of 1 Gm. procaine amide every three hours eliminated these irregularities and the medication was discontinued after five doses. Thirty-eight hours later (1/12/50) ventricular tachycardia recurred. The oral administration of 1.5 Gm. procaine amide established normal sinus rhythm in 45 minutes. Oral maintenance therapy, 1 Gm. every four hours, was continued for four days and during this interval the patient had no aberrant beats. Medication was then discontinued. Three weeks later the patient once again complained of palpitation and an electrocardiogram again showed that ventricular tachycardia had recurred (fig. 2*A*). Procaine amide was given intravenously and at the end of 200 mg. supraventricular beats were observed to interrupt the ventricular rhythm, and after a total of 400 mg. the ectopic ventricular beats disappeared (fig. 2*B*, *C*). The rhythm again became nodal in origin for about two hours and then became normal sinus. An oral maintenance dose of 0.5 Gm. every four hours was given for five months, and during this period the patient remained in normal rhythm. At the end of this period, the medication was discontinued and during the succeeding 10 months there has been no recurrence of the abnormal rhythm.

Case 7. E. R., a 54 year old white male, was admitted on Dec. 25, 1949 with a history of angina on effort for 3 weeks and sudden severe sticking substernal pain for 24 hours prior to admission. There was no history of any previous cardiovascular disease. Physical examination showed a temperature of 101 F.; pulse rate, 96; and blood pressure, 110/80; the patient was not in acute distress and there were no signs of congestive heart failure. Heart sounds were of good quality; no thrills or murmurs were noted. The electrocardiogram showed normal sinus rhythm and changes indicative of acute infarction of the anterior wall of the heart. Therapy included oxygen, sedation, anticoagulants and quinidine, 0.4 Gm. every four hours, as a prophylactic measure. Eight days later (1/2/50), after an entirely uneventful course, the quinidine was discontinued. It was instituted again 24 hours later (1/3/50), when the patient had many ventricular premature contractions (0.4 Gm. administered every four hours four times). The next day (1/4/50), sustained ventricular tachycardia was present and the quinidine was increased to 0.65 Gm. every two hours to a total of 8 Gm. a day. The next day (1/5/50), there was a temporary establishment of normal sinus rhythm but the aberrant rhythm reappeared on 1/6/50 and continued uninterrupted for six days despite daily dosage of 8 Gm. of quinidine. On 1/12/50, a total of 1.5 Gm. of procaine amide was administered intravenously in two doses 35 minutes apart without altering the rhythm. One half hour later an oral dose of 1.5 Gm. was given, and two hours later an additional Gm. was given. An electrocardiogram taken one half hour after the second dose showed that normal sinus rhythm was present. He remained on a maintenance dose of procaine amide, 1 Gm. every four hours (six doses daily) for four days, without any aberrant beats being observed. Medication was then discontinued and the patient had an uneventful convalescence from the myocardial infarction.

Case 8. H. F., a 78 year old white male, was admitted to the hospital on Feb. 6, 1950 with a history of nausea, vomiting and precordial pain

for one week. He had been hospitalized previously in 1947 because of anterior wall infarction, and again in March 1948 because of thrombophlebitis and pulmonary infarction. In October 1948 he had again been hospitalized because of precordial pain, nausea and vomiting. During four weeks of observation the findings were insufficient to establish the diagnosis of fresh myocardial infarction. Diagnosis on discharge was arteriosclerotic heart disease, coronary insufficiency and aneurysm of the left ventricle. For the four months prior to the last admission, the patient continued to receive digitalis and mercurial diuretics for his congestive heart failure. On admission, an electrocardiogram showed a ventricular tachycardia at a rate of 150 (fig. 3*A*, *B*, *C*, *D*). Quinidine sulfate, 0.2 Gm. orally every hour for sixteen doses (3.2 Gm.), had no effect on the rhythm. On 2/8/50 1 Gm. of procaine amide was given intravenously in five minutes, and directly after completion of the injection the ventricular tachycardia was abruptly terminated with the establishment of a supraventricular rhythm; the pacemaker was located at first in the auriculoventricular node, and then in the sinoauricular (fig. 3*E*, *F*, *G*, *H*). He was given 1 Gm. procaine amide orally every four hours for maintenance therapy but could not tolerate this dose because of nausea. The drug was therefore discontinued after two days. One week later paroxysmal ventricular tachycardia reappeared. He was given procaine amide orally 1 Gm. every hour and after the second dose was in normal sinus rhythm. Maintenance therapy (4 Gm. a day) was successfully continued for five weeks. Clinical findings and serial electrocardiograms confirmed the impression of fresh myocardial infarction. Patient was discharged after a lengthy hospitalization.

Case 9. J. B. was a 61 year old white male with a history of congestive heart failure controlled by digitalis and mercurial diuretics for two years. Though he gave no history of rheumatic fever, he had evidence of valvular deformities of both the mitral and aortic valves. Electrocardiograms in this period had revealed auricular fibrillation. On 3/8/50 digitalis medication was discontinued and in the next 10 days the patient gained 14 pounds and went rapidly into acute congestive heart failure. When examined on 3/18/50, he exhibited signs of pulmonary edema and a very rapid apical rate. An electrocardiogram showed the presence of ventricular tachycardia. After receiving 200 mg. procaine amide intravenously the ventricular rhythm ceased and auricular fibrillation with a ventricular rate of 130 supervened. The rhythm remained unchanged for the next 30 minutes. At the end of this period 0.8 mg. lanatoside C was given intravenously in 10 minutes. An electrocardiogram at the end of this injection again revealed ventricular tachycardia. Procaine amide therapy intravenously was again started but by the time that 150 mg. were injected, the rhythm changed to ventricular fibrillation and the patient expired. Postmortem examination revealed extensive rheumatic involvement of the aortic, mitral and tricuspid valves. An area of fresh pulmonary infarction was found in the left lung.

Case 10. J. W., a 60 year old white male, was known to have had hypertension for eight years. On Jan. 2, 1950 he was hospitalized because of severe precordial pain. Clinical and electrocardiographic findings substantiated the diagnosis of acute myocardial infarction. His course during three months of hospitalization and convalescence was entirely uneventful. One week after discharge, the patient noted increasing dyspnea on exertion and ankle edema. A maintenance dose of digitalis was prescribed and during the next week there was gradual improvement in the patient's condition. Suddenly, on the eighth day, the patient noted palpitation and weakness. Examination revealed a very rapid cardiac rate, and the patient was hospitalized. An electrocardiogram showed a ventricular tachycardia at a rate of 200. He was given 1 Gm. of procaine amide intravenously in divided doses, with the establishment of a nodal rhythm. Subsequent electrocardiograms and clinical findings indicated fresh myocardial damage. Ten days later (5/15/50), the patient again developed ventricular tachycardia during defecation. On this occasion 400 mg. procaine amide injected intravenously restored normal rhythm. During the succeeding six months of hospitalization the patient had six bouts of ventricular tachycardia, each of which was terminated by procaine amide, administered orally, intravenously or by both routes. Four of these bouts appeared to be precipitated by the use of various digitalis glycosides given because of appearance of signs of congestive heart failure. Two of these bouts occurred despite a high maintenance dose (6 Gm. a day) of procaine amide orally. At present (12/11/50), the patient is satisfactorily maintained on this oral maintenance dose, and his congestive heart failure is controlled by diet and mercurial diuretics.

Case 11. S. F., a 60 year old white male, was first admitted to the hospital on Jan. 24, 1950 complaining of left-sided chest pain. He had been treated for thrombophlebitis of the left leg which had developed five days prior to admission. He was hospitalized for pulmonary infarction. Subsequently, both clinical and laboratory findings indicated hepatitis which prolonged his stay in hospital for two months. He was discharged on 3/15/50. Two months later he was again hospitalized because of recurrent pulmonary infarction. On admission, an electrocardiogram showed ventricular tachycardia. He was given 1 Gm. procaine amide intravenously following which normal sinus rhythm was promptly reestablished. A bilateral

femoral vein ligation was performed the next day and the patient had an uneventful convalescence.

Case 12. W. B., a 65 year old white male with a 10 year history of hypertension and a left hemiplegia sustained in 1947, was admitted to the hospital on May 9, 1950 because of palpitation and syncope. An electrocardiogram revealed a ventricular tachycardia. Quinidine sulfate was given orally, 0.3 Gm. every two hours for six doses, with no change in the rhythm. At the end of this period, 0.8 mg. of lanatoside C was given intravenously every 12 hours for four doses with no effect on the rhythm. Procaine amide (1.2 Gm.) was given intravenously without any change in the rhythm. The patient then received 3 Gm. procaine amide orally in divided doses over a five hour period and the ventricular tachycardia ceased. The initial rhythm established after interruption of the tachycardia was idioventricular with complete auriculoventricular dissociation; subsequently normal sinus rhythm appeared. Evidence of fresh myocardial damage was lacking and the patient was discharged two weeks later.

Case 13. M. F., a 70 year old white woman, was hospitalized since 1947 because of a left hemiplegia and aphasia. On March 22, 1950 the patient complained of difficulty in voiding and was given 1 cc. of prostigmine (1:2000) subcutaneously. One hour later her blood pressure fell to 80/60 and the patient became cold and vomited. An electrocardiogram revealed ventricular tachycardia. The intravenous administration of 0.5 mg. of ouabain had no effect on the rhythm. One hour later the intravenous administration of 500 mg. of procaine amide promptly restored normal sinus rhythm. There has been no subsequent change in her clinical status.

Case 14. E. P., a 70 year old white woman with a history of hypertension for five years and diabetes mellitus for eight months, was hospitalized in 1949 for an acute myocardial infarction. She was admitted to the hospital again on Aug. 22, 1950 because of severe dyspnea. It was difficult to obtain an accurate history, but the patient had received digitalis (0.1 Gm.) for 10 days prior to admission because of increasing dyspnea and edema. On admission an electrocardiogram showed ventricular tachycardia. She was given 0.25 Gm. procaine amide intravenously without effect, but the second injection of the same amount 30 minutes later was followed by a change in rhythm to that of auricular fibrillation with premature ventricular contractions. Oral procaine amide was given, 0.25 Gm. every four hours. The patient was digitalized over the next 24 hours and the ventricular rate slowed to 112, but the auricles continued to fibrillate. Six hours later the patient spontaneously reverted to normal sinus rhythm. Digitalis and procaine amide were discontinued. Dyspnea disappeared and no other signs of congestive failure occurred during her convalescence.

Case 15. D. L., a 53 year old white woman with a history of extensive rheumatoid arthritis for 10 years, was hospitalized in September 1949 because of exacerbation of her arthritis. For five and a half months her clinical course was uneventful; on 3/2/50 her temperature rose suddenly to 104 F., apparently due to an ischiorectal abscess. The heart rate was found to be rapid (136) and an electrocardiogram revealed ventricular tachycardia. Quinidine by mouth for the next two days temporarily interrupted the tachycardia, but the patient became unable to tolerate further medication with quinidine. On the evening of 3/4/50, the injection of 0.5 Gm. of procaine amide intravenously was immediately followed by cessation of the ventricular tachycardia and oral maintenance therapy was started at this time. On the next day, the patient had two bouts of ventricular tachycardia, each controlled by an additional dose of 1 Gm. procaine amide orally. For the next 10 days, the patient received between 8 and 12 Gm. orally each day and maintained normal sinus rhythm with occasional premature ventricular contractions. The daily dose of 12 Gm. appeared to be toxic as evidenced by anorexia, nausea, vomiting and mental confusion. On 3/14/50, the patient again became febrile without obvious explanation and the procaine amide was discontinued. The fever persisted and the patient's condition deteriorated rapidly; she died on 3/17/50. There were no rhythmic abnormalities during the last three days of her life. Postmortem examination revealed old rheumatoid arthritis, extensive diffuse fibrinous pericarditis, and areas of collagen necrosis in the myocardium.

Summary

1. Procaine amide, an analogue of procaine, has been found to be effective in treating arrhythmias of ventricular origin.

2. In 54 patients with premature ventricular contractions, procaine amide administered orally or intravenously effectively suppressed the aberrant beats.

3. In 13 of 15 patients with ventricular tachycardia, the abnormal rhythm was terminated by intravenous or oral administration of procaine amide. Six had previously been treated with quinidine to toxicity without success.

4. Oral rather than intravenous administration is preferable, unless the patient is comatose or vomiting.

5. Procaine amide did not establish normal rhythm in 24 patients with chronic auricular flutter and fibrillation. In 2 cases of recent auricular fibrillation, normal sinus rhythm followed the use of the drug.

Acknowledgments

The authors wish to express their indebtedness to the many physicians and hospitals in New York City and vicinity who notified us of the existence of patients with cardiac arrhythmias and who have kindly permitted us to report on treatment with procaine amide. It would have been impossible to accumulate this series of cases without their cooperation.

References

[1] Burstein, C. L.: The utility of intravenous procaine in the anesthetic management of cardiac disturbances. Anesthesiology **10:** 133, 1949.

[2] Clark, B. B.: Personal communication.

[3] Mark, L. C., Kayden, H. J., Steele, J. M., Cooper, J. R., Berlin, I., Rovenstine, E. A., and Brodie, B. B.: The physiological disposition and cardiac effects of procaine amide. J. Pharmacol. & Exper. Therap. **102:** 5, 1951.

[4] Rosenberg, B., Kayden, H. J., Lief, P. A., Mark, L. C., Steele, J. M., and Brodie, B. B.: Studies on diethylaminoethanol. J. Pharmacol. & Exper. Therap. **95:** 18, 1949.

[5] Rovenstine, E. A., and Joseph, S.: Personal communication.

Electron Micrograph of Plasma Lipoprotein Molecule (S_f 10-20)

As part of a current study of cardiovascular disease, electron microscope photographs were obtained which confirm the presence of a lipoprotein molecule comparable in size to the S_f 10-20 molecules found with the ultracentrifuge.

By JOHN J. PRENDERGAST, M. D., AND D. MAXWELL TEAGUE, PH. D.

BECAUSE of the interest in the blood lipoprotein which Gofman and coworkers[1, 2] have shown to be frequently associated with atherosclerosis, we are submitting an electron micrograph (fig. 1) showing what we believe to be individual molecules of the protein. Gofman has presented evidence to show that there is a high molecular weight cholesterol-protein complex present in the blood of atherosclerotic individuals, especially those with coronary heart disease. This lipoprotein complex has been separated with the ultracentrifuge, since it has a rate of 10 to 20 Svedberg flotation units compared with 3 to 7 units for the normal cholesterol-protein molecule.

The specimen examined with the electron microscope was a concentrate prepared by Dr. Lena Lewis, Research Division of the Cleveland Clinic; it contained both the normal S_f 3–7 and the abnormal S_f 10–20 fractions. The protein, suspended in a 10 per cent saline solution, was diluted 1:1000 with distilled water and a drop was placed on a previously prepared film of Parlodion. After drying, the film was gently submerged in distilled water to dissolve the salt, redried, and vacuum-shadowed with palladium.[3, 4]

Careful measurements of what appeared to be single particles were made on several electron micrographs. The particle size distribution is presented in figure 2, and indicates a mean diameter of 25 mμ. The minor peak at 40 mμ probably represents agglomerates of two molecules.

Gofman estimates that the lipoprotein has a density of 1.04 and a molecular weight of

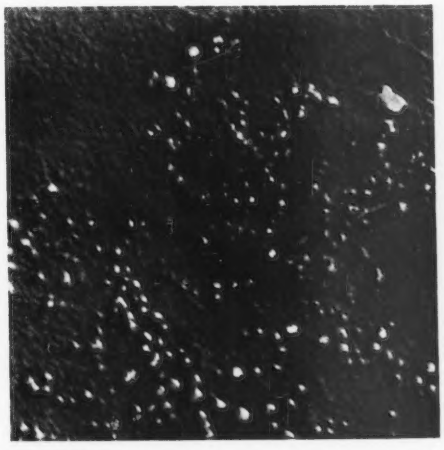


FIG. 1. Molecules of the lipoprotein presumed to be associated with atherosclerosis. Magnification 25,000.

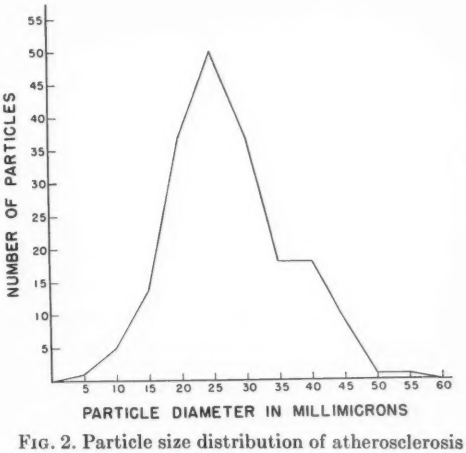


FIG. 2. Particle size distribution of atherosclerosis lipoprotein. Particles were measured from several representative electron micrographs. The mean diameter is 25 mμ.

From the Medical and Engineering Departments, Chrysler Corporation, Detroit, Mich.

3,000,000.[2] Assuming that the molecule is in a coiled (spherical) form, this would be equivalent to a diameter of 21 mμ. The electron micrographs thus permit visualization of the cholesterol-containing lipoprotein, and support the previous estimate as to size.

REFERENCES

[1] Gofman, J. W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., Herring, V., and Lyon, T. P.: The role of lipids and lipoproteins in atherosclerosis. Science **111:** 166, 1950.

[2] —, Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A., and Strisower, B.: Blood lipids and human atherosclerosis. Circulation **2:** 161, 1950.

[3] Williams, R. C., and Wyckoff, R. W. G.: Applications of metallic shadow-casting to microscopy. J. Appl. Physics **17:** 23, 1946.

[4] Williams, R. C., and Backus, R. C.: The electron-micrographic structure of shadow-cast films and surfaces. J. Appl. Physics **20:** 98, 1949.

Lipoproteins, Liver Disease, and Atherosclerosis

By F. T. Pierce, M. D., and J. W. Gofman, M. D., Ph. D.

The blood levels of the S_f 10–20 class of giant cholesterol-bearing lipoproteins were studied in a group of patients with cirrhosis. No significant difference was observed between the blood levels of this group and of a similar group of normal individuals of corresponding age and sex. This observation suggests that patients with cirrhosis are as susceptible to atherosclerosis as the general population.

A CLINICAL impression has long been in existence indicating a lesser degree of atherosclerosis in persons with Laennec's cirrhosis. From an analysis of an extensive autopsy experience, Wilens[1] has presented substantial evidence that there is no significant difference in the extent of atherosclerosis in cirrhotics as compared with other individuals of comparable status and of equivalent age and sex distribution. In view of this controversy it was of interest to investigate in cirrhotics the blood levels of those giant cholesterol bearing lipoproteins, recently shown to be related to atherosclerosis in man.[2] The evidence relating such molecules to atherosclerosis involved a demonstration of the occurrence of a higher frequency of elevated levels of S_f 10–20 lipoproteins in patients with myocardial infarcts and in certain diseases predisposing to atherosclerosis (nephrosis, xanthoma tuberosum, diabetes, myxedema).

Thirty-four patients were studied, ranging in age from 17 to 72 years, including both males and females. The blood serum was analyzed for lipoproteins of the S_f 10–20 class ultracentrifugally by the technic described.[2,3] Table 1 summarizes the pertinent clinical and laboratory data and the S_f 10–20 lipoprotein levels. Figure 1 is a plot comparing the distribution of levels in the cirrhotic with the levels in clinically normal individuals of comparable age and sex distribution. It is evident that cirrhotics have at least as high, and possibly even higher levels of the S_f 10–20 class of molecules as do clinically normal individuals of corresponding age and sex. However, the difference between these two groups is not significant so no further refinement can be obtained from the data of figure 1.

No correlation between the levels of the S_f 10–20 class of molecules and any of the clinical or laboratory findings recorded in table 1 could be established.

It is of interest that both of 2 male patients, 17 and 21 years of age, in the active phase of infectious hepatitis showed very high levels of S_f 10–20 lipoproteins. This is in contrast to the rarity of such high levels in normals of this same age group. While this may indicate a disturbance of lipoprotein metabolism during acute parenchymal liver disease, a larger series must be studied to establish the significance of this observation.

Since the level of the S_f 10–20 class of molecule has been correlated with atherosclerosis and since the level of such molecules is not significantly different in patients with chronic hepatitis as compared with "normals," one would anticipate that such patients might show the same degree of atherosclerosis as found in the general population. These findings would support Wilen's observations indicating that patients with chronic hepatitis are not protected against atherosclerosis.

Summary

The blood levels of the S_f 10–20 class of molecules measured in 32 patients with chronic

From the Division of Medical Physics, Donner Laboratory and the Radiation Laboratory, University of California, Berkeley, Calif.

This work was supported in part by the United States Public Health Service and the Atomic Energy Commission.

During the course of the study, one of us (F. T. P.) was a Postdoctoral Fellow of the National Cancer Institute, United States Public Health Service.

TABLE 1.—*Clinical Data on the Cirrhotic Patients Used in This Study**

Case No.	Sex	Age	Ascites	Liver	Spleen	Icteric Index	Cephalin Flocculation	Albumin-Globulin Ratio	Thymol Turbidity	RBC Millions	Hemoglobin	Diagnosis	Sf10-20	Remarks
				cm.	*cm.*		*48 hours*	*Gm. per cent*			*Gm. per cent*		*mg. per cent*	
1	M	40	yes			2.1	++++	2.5/3.3		2.5	8.0	Cirrhosis with hepatoma (biopsy)	11	Expired 2 days after test
2	M	48	yes			8.6	++++	2.9/3.2	2.1	3.6	10.0	Cirrhosis	37	Expired 12 days after test
3	M	27	no	3		12.0	+	2.6/3.3	3.8	3.3	10.0	Cirrhosis	81	Expired 6 days after test
4	M	57	no	3		47.0	+	2.8/2.9	2.3	3.1	7.0	Cirrhosis	95	
5	F	49	yes	5		21.0	++++	2.6/2.8	8.3	2.7	7.8	Cirrhosis	68	
6	F	42	no			44.0	++++	2.9/2.9	3.3	3.7	12.3	Severe fatty infiltration of liver (autopsy)	110	Expired 5 days after test
7	F	43	no	5	4	25.0	++++	3.0/3.5			10.0	Cirrhosis and psoriasis	73	
8	F	49	yes	4		55.0	+++	3.0/3.9	2.5	3.0	7.0	Cirrhosis	31	
9	M	43	yes			9.0	+++	2.6/2.9			9.5	Cirrhosis	42	
10	M	65	no	4		5.0	+	2.3/3.7		2.7	9.0	Cirrhosis (biopsy)	53	Expired 10 days after test
11	M	55	no	3		82.0	++++	2.5/4.7	8.7	3.9	14.0	Cirrhosis	50	Expired 13 days after test
12	M	48	no	3		80.0	++++	3.1/5.0	8.0	4.7	12.0	Cirrhosis	26	
13	F	35	no	12	5	56.0	+++	2.3/3.4	4.4		9.0	Cirrhosis	132	
14	F	72	yes	3		15.2	±	2.8/1.9		4.6	15.0	Cirrhosis (biopsy)	7	Expired 18 days after test
15	F	54	yes	5		6.1	++++	2.8/3.0		4.1	12.5	Cirrhosis	35	Expired 12 days after test
16	M	47	yes	2		32.0	++	3.2/4.1		2.9	11.0	Cirrhosis (biopsy)	64	Expired 32 days after test
17	M	55	yes	4		12.3	+	2.9/2.8	10.5		8.4	Cirrhosis	48	
18	M	60	no	4		13.2	++++	2.8/3.3	3.1	3.7	11.0	Cirrhosis	53	Expired 5 days after test
19	M	52	no	3			0		1.0	3.3	9.8	Cirrhosis (biopsy)	64	
20	M	47	no			60.0	+++	2.7/4.0	12.0		11.0	Cirrhosis (biopsy)	26	
21	F	69	no	2							12.3	Cirrhosis (biopsy)	33	
22	M	55	yes	3		4.0	+++	2.2/3.3	8.0		10.5	Cirrhosis	73	
23	M	67	no		1		++				12.6	Cirrhosis (biopsy)	84	
24	F	70	yes			39.0		2.9/3.6			11.0	Cirrhosis	29	Expired 120 days after test

TABLE 1.—*Concluded*

Case No.	Sex	Age	Ascites	Liver	Spleen	Icteric Index	Cephalin Floculation	Albumin-Globulin Ratio	Thymol Turbidity	RBC Miliions	Hemoglobin	Diagnosis	Sf 10-20	Remarks
				cm.	*cm.*		*48 hours*	*Gm. per cent*			*Gm. per cent*		*mg. per cent*	
25	M	50	yes	2								Cirrhosis	53	
26	M	46	yes	2		8.0	++++	2.4/3.4	10.0		13.0	Cirrhosis	66	Expired 4 days after test
27	M	54	yes	3		42.0	++	2.6/1.8		4.5	11.5	Cirrhosis (biopsy)	48	
28	F	53	yes			21.0	+++	2.5/3.7			13.0	Cirrhosis	70	
29	M	65	yes	6		14.0	++++	2.8/2.7		2.3	8.9	Cirrhosis	18	
30	M	72	yes			41.0	++++	2.1/3.9		4.1	14.5	Cirrhosis	51	
31	M	55	no	4		8.0	++	3.4/2.6			13.0	Cirrhosis	33	
32	M	39	yes	3		21.0	++++	2.0/4.5		3.1	14.0	Cirrhosis	26	Expired 41 days after test
33	M	21	no			47.5	0	4.0/3.5	5.7	5.6	15.0	Infectious hepatitis	150	
34	M	17	no			15.2	++++	3.7/2.7		5.3	13.0	Infectious hepatitis	68	

* Two patients with infectious hepatitis are included.

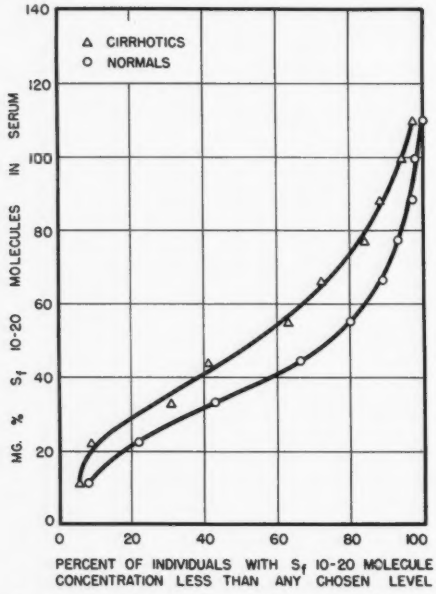


FIG. 1. Diagram illustrating the levels of S_f 10–20 molecules in patients with cirrhosis and normal individuals of corresponding age and sex.

hepatitis were at least as high as, and possibly higher than, those found in clinically normal individuals of corresponding age and sex. These data offer no support for the contention that chronic hepatitis is accompanied by a lesser degree of atherosclerosis than is seen in the general population.

Preliminary data indicate elevated S_f 10–20 levels in the active phase of acute hepatitis.

ACKNOWLEDGMENTS

The authors wish to thank the Department of Public Health, San Francisco Hospital (J. C. Geiger, Director), and the Alameda County Hospitals for their generous cooperation in this study.

REFERENCES

[1] WILENS, L.: The relationship of chronic alcoholism to atherosclerosis. J. A. M. A. **135:** 1136, 1947.

[2] GOFMAN, J. W., JONES, H. B., LINDGREN, F. T., LYON, T. P., ELLIOTT, H. A., AND STRISOWER, B.: Blood lipids and human atherosclerosis. Circulation **2:** 161, 1950.

[3] GOFMAN, J. W., LINDGREN, F. T., AND ELLIOTT, H. A.: Ultracentrifugal studies of lipoproteins of human serum. J. Biol. Chem. **179:** 973, 1949.

The Effect of Carbon Tetrachloride Poisoning on Serum Lipoproteins Associated with Atherosclerosis

By F. T. PIERCE, M. D., AND J. W. GOFMAN, M. D., PH. D.

Carbon tetrachloride was injected into normal rabbits and the levels of S_f 3–12, 12–20, and 20–40 classes of lipoproteins were determined during the course of these injections. A marked increase in concentration of these three classes of lipoproteins occurred during the administration of carbon tetrachloride. After the cessation of this drug, the lipoproteins of largest S_f rate returned to normal first with those of lower S_f rate returning to normal levels in the order of decreasing S_f rates. Rabbits fed cholesterol were also injected with carbon tetrachloride, and their lipoproteins continued to increase after cessation of this drug.

ULTRACENTRIFUGAL studies reported by Gofman and co-workers[1] have revealed certain lipoproteins in the serum of rabbits and humans that are associated with the development of atherosclerosis. Studies now in progress in this laboratory are directed toward elucidation of the factors responsible for the maintenance of the blood levels of the various lipoproteins. The probable role of the liver as an important organ involved in lipid metabolism prompted the study of the effect of impaired liver function on the levels of the lipoproteins in the blood. Carbon tetrachloride (CCl_4) has long been known for its ability to produce a fatty liver and eventual hepatic cirrhosis.[2] This agent has also been shown to be capable of producing an elevation in the total serum cholesterol levels in the rabbit.[3] It was felt, therefore, that the nature of lipid transport (in terms of the lipoproteins involved) in animals treated with carbon tetrachloride might provide information as to certain factors, at least, involved in maintaining blood lipoprotein levels.

The normal rabbit shows ultracentrifugally the presence of lipoproteins characterized by flotation rates in the range of S_f 3–12.* A few apparently normal rabbits show, in addition, *very low* concentrations of lipoproteins with flotation rates S_f 12–20. During cholesterol feeding experiments rabbits in general show increases in the S_f 3–12 components, followed by the development of very high levels of other lipoproteins of flotation rates greater than 12 S_f units and up to flotation rates over 100 S_f units. In general the molecules of higher S_f rates develop following a prior increase in those of the lower S_f rates, for example, appreciable concentrations of S_f 20–40 lipoproteins appear only after significant levels of S_f 12–20 components have appeared. These previous studies have indicated that the extent of atherosclerosis developing in such rabbits parallels the level of the S_f 12–30 class† of lipoproteins. These studies also indicated that the molecules

From the Division of Medical Physics, Donner Laboratory and the Radiation Laboratory, University of California, Berkeley, Calif.

This work was supported in part by the United States Public Health Service and the Atomic Energy Commission. During the course of the work one of us (F. T. P.) was a Postdoctoral Fellow of the National Cancer Institute, United States Public Health Service.

* One S_f unit represents a flotation rate of 1×10^{-13} cm. per sec. per dyne per Gm. in a sodium chloride solution of density 1.063 Gm. per cc. at 26 C. The lipoproteins were previously isolated from other serum proteins (and high density lipoproteins) by a preparative ultracentrifugation. Spinco Model L and Model E ultracentrifuges were used.

† In the original report by Gofman and associates,[1] the normally occurring lipoproteins in rabbits migrated with rates less than 10 S_f units. However, since some rabbits are now known to have normal components migrating at rates up to 12 S_f units, the class of "abnormal" lipoproteins is so chosen as always to exclude the normally occurring lipoproteins.

of successively higher flotation rates are of successively lower densities and of lower protein content per molecule. It is entirely possible that the lipoprotein molecules of higher S_f values than 12 occur normally in the lipid metabolic pathways, but are maintained at such low levels in the blood as to escape resolution ultracentrifugally or to be demonstrable at very low levels. The studies reported below suggest the nature of the interrelationships of several of these lipoprotein classes involved in lipid transport.

TABLE 1.—*Changes of Cholesterol and Lipoproteins in Rabbits Injected with Carbon Tetrachloride**

Rabbit	Day from Start of Experiment	Lipoproteins, mg. %			Cholesterol, mg. %		
		3-12	12-20	20-40	Free	Total	F:T
#1	0	136	22	2	19	87	0.23
	14	198	59	151	145	350	0.41
	28	253	77	106	—	—	—
	42	242	84	106	64	137	0.47
	56	171	149	50	39	116	0.34
	70	209	24	7	28	116	0.24
#2	0	59	4	2	10	62	0.16
	14	198	70	176	139	344	0.40
	28	268	92	139	—	—	—
	42	172	59	26	46	111	0.41
	56	138	85	22	39	111	0.35
	70	128	29	0	19	83	0.23
#3	0	51	57	4	14	63	0.23
	14	169	88	132	120	312	0.39
	28	308	62	136	—	—	—
	42	239	103	187	88	194	0.45
	56	77	160	19	27	139	0.20
	70	150	44	22	16	68	0.24
#4	0	37	22	0	5	56	0.09
	14	327	92	180	111	281	0.40
	28	272	88	150	127	320	0.40
	42	235	84	31	43	132	0.33
	63	79	9	0	12	59	0.20

* Last injection given on day 39.

Arbitrarily, the ultracentrifugal diagrams have been analyzed into three broad classes of lipoproteins, the S_f 3–12 group (which include those normally appearing in rabbits), the 12–20 class, and the 20–40 class.

Two general types of experiments were performed. In one group, the effect of carbon tetrachloride injections alone on the blood lipoprotein pattern was determined. In a second group, the combined effect of carbon tetrachloride injection plus cholesterol feeding was studied. Female rabbits, weighing between 2 and 4 Kg., of the New Zealand white strain were used in all experiments. Seven of 14 animals originally started on carbon tetrachloride injections survived the entire 10 week period of study. Three of these were fed cholesterol throughout the entire period and 4 were on a normal diet (Albers family style rabbit pellets). The cholesterol food was prepared by dissolving 1 Gm. of cholesterol in 8 cc. Wesson Oil, which was then thoroughly mixed with 100 Gm. rabbit pellets.

After a control blood specimen was drawn, carbon tetrachloride was injected subcutaneously using a dose of 1 cc. per Kg. These injections were given twice a week for five and one-half weeks (a total of 11 injections). Blood specimens were obtained at weekly intervals for 10 weeks at which time the experiment was terminated and the animals sacrificed.

Serum was analyzed ultracentrifugally for lipoproteins and by the Schoenheimer-Sperry method for free and total cholesterol.*

RESULTS AND DISCUSSION

A summary of the results obtained is given in tables 1 and 2. The data of table 1, which summarize the effect of carbon tetrachloride alone on the serum lipoproteins, show a consistent trend of events in all 4 animals. The serum level of the normally occurring S_f 3–12 class of lipoprotein molecules is invariably increased in concentration during the course of carbon tetrachloride injections. Coincident with this increase is the appearance of considerable levels of both the S_f 12–20 and 20–40 classes of molecules. This sequence of changes is the same as that found in a rabbit fed cholesterol. In other words, carbon tetrachloride in rabbits not fed cholesterol is capable of mimicking the lipid and lipoprotein alterations of the serum found when rabbits are fed cholesterol. This represents the synthesis of these "abnormal" giant cholesterol-bearing molecules (of the S_f

* The authors wish to thank David Colman and Donald Fulghum for the cholesterol determinations.

12–20 and 20–40 classes) from endogenous sources alone. Parallel with this rise in these three classes of lipoproteins during carbon tetrachloride administration is a concomitant rise in serum cholesterol levels and an increase in the ratio of free to total cholesterol.

When the carbon tetrachloride injections were stopped, an interesting sequence of events occurred during the recovery of the animals from carbon tetrachloride poisoning. As can be seen from table 1, the S_f 20–40 class of molecules returned to relatively low levels within two weeks of the last injection of carbon tetrachloride. At this time the concentration of the S_f 3–12 and 12–20 classes was still elevated, and, in some instances, the S_f 12–20 class showed a moderate increase in concentration. By four weeks after the last injection of carbon tetrachloride, however, the S_f 12–20 class of molecules returned to approximate control levels, although the S_f 3–12 class was still considerably above the original levels before carbon tetrachloride treatment was begun. At the end of the experiment the concentration of the S_f 3–12 class had begun to decrease toward control levels.

During the recovery phase, the ultracentrifugal photographs portray the disappearance of molecules of high S_f value first followed by those of progressively lower S_f value. (See fig. 1.) For example, within the S_f 12–20 class, the species at the higher S_f range decrease before those of the lower S_f range do.

None of these non-cholesterol fed animals showed any atherosclerosis in spite of the transient elevation of the S_f 12–20 and 20–40 classes of molecules during carbon tetrachloride administration. However, the concentration of the S_f 12–40 molecules in rabbits fed cholesterol and developing atherosclerosis is considerably higher than was observed in this experiment. Hence macroscopic atheroma would not be expected in the carbon tetrachloride injected rabbits in the short experimental period studied.

Table 2 shows the sequence of events in a carbon tetrachloride injected rabbit being fed cholesterol. The increase in the three classes of molecules analyzed occurs actually more slowly than in a normal rabbit fed cholesterol at the dosage used. However, these animals were rapidly losing weight during the injections and ate very little of the food presented to them. After the last injection of carbon tetrachloride, large quantities of the S_f 12–20 and 20–40 classes of lipoproteins developed very rapidly while the S_f 3–12 class remained the same or diminished. The serum cholesterols of this group of animals similarly increased during the carbon tetrachloride injections. After the last injection of carbon tetrachloride, the cholesterol levels increased rapidly to very high levels as the animals began to eat more food and consequently increased their intake of cholesterol. All these animals fed cholesterol as well as injected with carbon tetrachloride and which survived the 10 week period showed atherosclerosis.

It should be pointed out that normal untreated rabbits not fed cholesterol consistently show the distribution of lipoproteins as illu-

TABLE 2.—*Changes of Cholesterol and Lipoproteins in Rabbits Injected with Carbon Tetrachloride and Fed Cholesterol.**

Rabbit	Day from Start of Experiment	Lipoproteins, mg. %			Cholesterol, mg. %		
		3-12	12-20	20-40	Free	Total	F:T
#5	0	24	11	0	7	46	0.16
	14	70	11	66	136	427	0.32
	28	77	264	418	—	—	—
	42	385	154	374	350	660	0.53
	56	396	495	869	444	907	0.49
	70	77	638	1892	478	1700	0.28
#6†	0	55	40	31	9	49	0.18
	14	490	154	160	—	—	—
	28	787	314	303	—	—	—
	42	666	281	281	508	642	0.79
	56	550	1078	704	534	753	0.71
	70	253	>1749	979	680	1971	0.35
#7	0	24	26	2	8	51	0.16
	14	297	92	154	181	450	0.40
	28	479	165	270	—	—	—
	42	803	198	193	203	408	0.50
	56	363	220	121	119	297	0.40
	70	396	1353	561	402	1436	0.28

* Last injection given on day 39.

† Given no CCl_4 from the second to the third week because of extreme jaundice and cachexia. This was the only animal which became jaundiced.

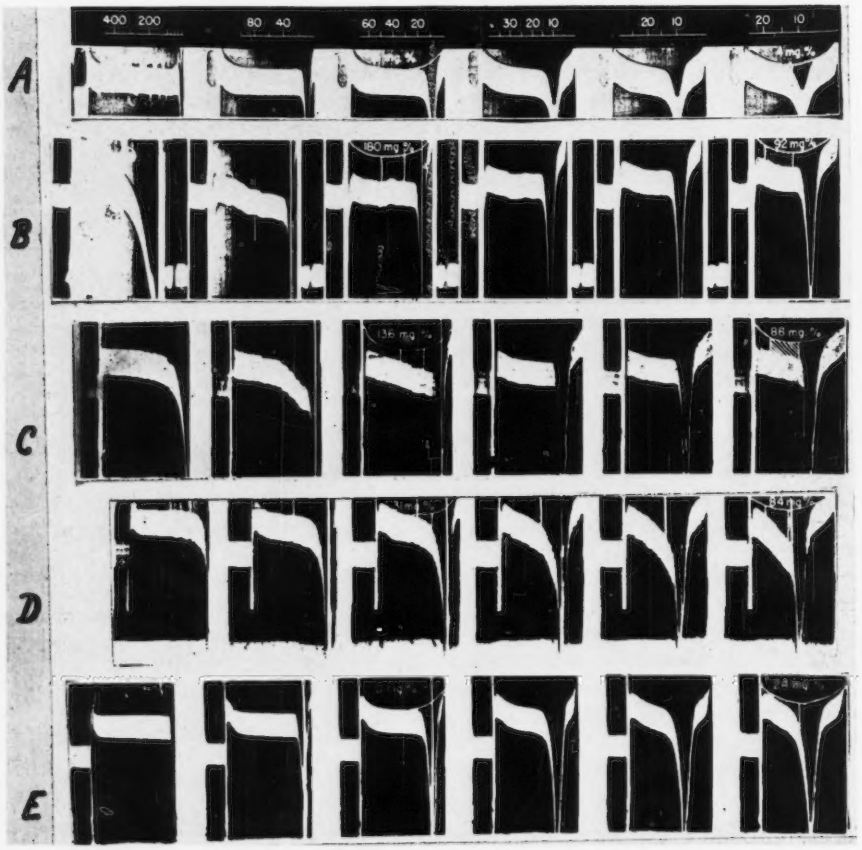


FIG. 1. *A*. Ultracentrifugal photographs showing the flotation of low density lipoproteins of a normal rabbit. Each frame is ruled for calculation of the S_f rate of any peak appearing in that frame. In this and all the other series of photographs, successive frames are at 0, 6, 12, 22, 30, and 38 minutes after the ultracentrifuge rotor has reached full speed (52,640 revolutions per minute). Consequently, these S_f markings can be used on corresponding frames in all the series of photographs below. In this pattern, the low density lipoproteins from 5 cc. of serum were concentrated into 1 cc. by preparative ultracentrifugation and then analyzed in the ultracentrifuge as described above.

B. Flotation pattern of low density lipoproteins of a rabbit after two weeks of carbon tetrachloride injections, showing markedly increased levels of lipoproteins. The low density lipoproteins from 3 cc. of serum were concentrated into 1 cc. in this pattern. Consequently, the increase in lipoproteins is 67 per cent greater than represented by these photographs when compared with the pattern above.

C. Flotation pattern of low density lipoproteins from a rabbit one week after the cessation of carbon tetrachloride injection. This shows the return toward normal levels of the lipoproteins of S_f 20–40 class, with those lipoproteins of higher S_f value disappearing first. The lipoproteins of 3 cc. serum were concentrated into 1 cc. in this pattern.

D. Flotation pattern of low density lipoproteins from a rabbit two weeks after the cessation of carbon tetrachloride injections. The molecules of the S_f 20–40 class are greatly reduced in concentration, and those of the S_f 12–20 class are disappearing with those of higher S_f value disappearing first. The lipoproteins of 5 cc. of serum were concentrated into 1 cc. in this pattern.

E. Flotation pattern of low density lipoproteins from a rabbit four weeks after the cessation of carbon tetrachloride. Molecules of the S_f 20–40 and 12–20 classes have returned almost to normal levels and the 3–12 class is beginning its return to normal levels. The lipoproteins of 5 cc. of serum were concentrated into 1 cc. in this pattern.

strated in day 0 (control levels) in the rabbits shown in tables 1 and 2. Repeat samples drawn from a normal rabbit show very little variation from week to week. Consequently the experimental changes reported are in marked contrast to these consistent low levels.

Rabbits injected with alloxan also show a transitory rise of serum cholesterol[4] and of the S_f 3–12, 12–20 and 20–40 and even higher classes of lipoproteins.[5] This substance produces acute liver damage with the development of a fatty liver. In rabbits fed cholesterol, fatty livers are also produced. The increase, often to very high level, in these various classes of lipoproteins which may be present normally in very small quantities may be a reflection of the inability of the damaged liver to handle endogenous or exogenous cholesterol (and other lipids) in the synthesis of the "normal" protein-lipid complexes and in their degradation, by whatever means it occurs. However, the more widespread toxic effects of carbon tetrachloride render it impossible to exclude involvement of other organ systems in the lipoprotein metabolic abnormality.

The changes in free:total cholesterol ratios which occur parallel with the rise and fall in level of the S_f 12–20 and S_f 20–40 classes of lipoproteins provide some information relative to internal structural features of the various lipoproteins. In the animals receiving carbon tetrachloride injections only, the free:total ratio is less than 0.25 at the outset when the lipoproteins present are predominantly in the S_f 3–12 class. As the lipoproteins of the S_f 12–20 and 20–40 classes appear in progressively increasing concentration, the free:total ratio rises significantly. This shows that these classes of lipoproteins differ structurally from the S_f 3–12 class in containing a higher proportion of the cholesterol of the molecule in the nonesterified state as fractionated by the Schoenheimer-Sperry method. These data are in harmony with the data on human lipoproteins which show progressively higher free:total ratios with increasing S_f rate of the lipoprotein species.[6]

Summary

1. Normal and cholesterol fed rabbits were injected with carbon tetrachloride and their serum cholesterol levels and lipoproteins of the S_f 3–12, 12–20, and 20–40 classes measured during and after the injections.

2. Carbon tetrachloride produced a marked increase above control levels in all classes of lipoproteins and in cholesterol in the non-cholesterol-fed rabbit. These substances gradually decreased to control levels after the cessation of carbon tetrachloride injections. No macroscopic atherosclerosis developed in this non-cholesterol fed group of animals as was predicted by the relatively low concentration of the S_f 12–40 class of molecules present for this limited amount of time.

3. In the cholesterol fed rabbit, cholesterol and all classes of lipoproteins increased during carbon tetrachloride injections but continued to increase after the cessation of carbon tetrachloride injections. Very large quantities of serum lipoproteins and cholesterol developed by the end of 10 weeks, and at this time all animals had developed atherosclerosis.

4. The data suggest that the increase in the normally occurring S_f 3–12 class and the appearance of high concentrations of S_f 12–20 and 20–40 classes of lipoproteins may occur as a result of impaired function of the degradation and synthetic system (possibly in the liver) involved in the metabolism of these molecules.

REFERENCES

[1] Gofman, J. W., Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A., and Strisower, B.: Blood lipids and human atherosclerosis. Circulation **2:** 161, 1950.

[2] Lamson, P. D., and Wing, R.: Early cirrhosis of the liver produced in dogs by carbon tetrachloride. J. Pharmacol. & Exper. Therap. **29:** 191, 1926.

[3] Dervillee, P., Castagnol, R., and Chomereau-Lamotte, F.: Les variations de la cholestérolémie chez le lapin après administration de tetrachlorure de carbone en ingestion. Compt. rend., Soc. de biol. **127:** 61, 1938.

[4] Duff, G. L., and Wilson, D. C., quoted in Duff, G. L., and McMillan, G. C.: The effect of alloxan diabetes on experimental cholesterol atherosclerosis in the rabbit. J. Exper. Med. **89:** 611, 1949.

[5] Pierce, F. T., Gofman, J. W., and Lindgren, F. T.: To be published.

[6] Lindgren, F. T.: Unpublished observations.

Observations with Radioactive Hydrogen (H^3) in Experimental Atherosclerosis

by MAX W. BIGGS, M. D., AND DAVID KRITCHEVSKY, PH. D.

Tritium has been used to study certain aspects of endogenous and exogenous cholesterol metabolism in normal and hypercholesterolemic rabbits. Special attention is given to turnover studies on aortic cholesterol. Labeled cholesterol has been used for feeding experiments and the subsequent rise of serum cholesterol specific activity has been investigated.

GOFMAN and co-workers[1] have made observations on the mode of transport of cholesterol in lipoprotein molecules in serum, and have presented evidence to link the appearance of certain of these lipoprotein molecules in human serum with the development of atherosclerosis. A general study of these molecules has been undertaken in this laboratory, directed toward gaining a fuller knowledge of their origin, function and fate, and thus clarifying their position in the pathogenesis of atherosclerosis. Cholesterol metabolism necessarily occupies a position of prominence in this study. Lipoprotein molecules similar to those occurring in human serum are to be found in great concentration in the hypercholesterolemic rabbit laying down atheromas, and for this reason the rabbit has been selected for this preliminary study. This report will present some observations on the fate of exogenous and endogenous cholesterol in normal and hypercholesterolemic rabbits.

Rittenberg and Schoenheimer[2] showed that in vivo cholesterol synthesis incorporates hydrogen from the body water into the cholesterol molecule, and Bloch and Rittenberg[3] prepared deuterium-labeled cholesterol. Thus an opportunity to study certain aspects of endogenous and exogenous cholesterol metabolism is afforded. Tritium has been used in this work for the hydrogen label; tritium-labeled cholesterol was prepared[4] using the method of Bloch and Rittenberg. The specific activity of this labeled cholesterol so prepared was not altered by refluxing with 30 per cent potassium hydroxide in 50 per cent alcohol for eight hours followed by 24 hours of standing at room temperature, indicating a stable tritium linkage in the tracer compound in the sense that exchange of tritium atoms for hydrogen atoms does not occur in an aqueous solution. Fukushima and Gallagher[5] have shown that in deuterium-labeled cholesterol prepared in this way 46 per cent of the total label is to be found in the vicinity of the Δ^5-3-hydroxyl system and that the other 54 per cent is concentrated in the isopropyl group of the isooctane side chain. Interpretation of results obtained with hydrogen-labeled cholesterol must be made cognizant of the fact that the metabolism of cholesterol hydrogen is being observed.

The specific activity of tissue cholesterol was determined as follows: All samples were carefully stripped of macroscopic fat, and a weighed, wet tissue sample was refluxed in 30 per cent potassium hydroxide in 50 per cent alcohol for eight hours. The alcohol was partially removed and the cholesterol extracted with ether. The ether extract was washed with water, dried with sodium sulfate, and evaporated to dryness. The residue was taken up in hot alcohol and made up to a known volume. An aliquot of this solution was taken for cholesterol analysis according to the method of Schoenheimer-Sperry as modified by Sobel and Mayer.[6] A second aliquot was taken at the same time for specific activity determination. A known amount of carrier, approximately 100 mg. of cholesterol, was usually added at this point. This mixture was warmed and an excess of digitonin, 1 per

From the Donner Laboratory of Medical Physics, University of California, Berkeley, Calif.

During the course of this study, one of us (M. W. B.) was Atomic Energy Commission Postdoctoral Research Fellow, and Research Fellow of the University of California.

cent in 80 per cent alcohol, was added. The digitonide was washed with 95 per cent alcohol, acetone-ether (1:2), and with anhydrous ether. The sample was dried in a 75 degree oven for 24 hours.

The dried cholesterol digitonide samples were burned and the water formed trapped in a dry ice–acetone bath. Hydrogen was generated from this water sample with lithium aluminum hydride ($LiAlH_4$) to fill an ionization chamber. The activity of the tritium-hydrogen mixture was measured with a vibrating reed electrometer. The specific activity of the tissue cholesterol was calculated employing a molecular weight of cholesterol digitonide equal to 1615, empirical formula of $C_{83}H_{138}O_{30}$. The specific activities as recorded refer to the measured electrometer drift per unit time of a unit volume of cholesterol hydrogen or water hydrogen as the case may be. With the filling system and technic employed, the standard deviation in the measurement of specific activity of a standard water sample was ±4 per cent; the standard deviation in the determination of tissue cholesterol specific activity was ±7 per cent.

Part 1

To obtain data on the turnover rates of cholesterol in various tissues in the normal rabbit, particularly aorta, 7 rabbits, 3 female and 4 male animals, were selected. These animals received an initial priming subcutaneous injection of tritium-water followed by a smaller daily injection designed to keep the specific activity of the body water as near a steady state as possible. Periodic blood samples were taken at from one to four day intervals for determination of the body water specific activity. The specific activities found were plotted against time and an average determined for each animal. The blood samples were taken eight hours after the preceding tritium-water injection. It had been shown in animal 1, by making six body water specific activity determinations in the first six hours and then two more at 12 and 24 hours after a single tritium-water injection, that the specific activity reached an equilibrium value in about four hours. A sample taken eight hours after the labeling injection approximated the average value for the day. Samples of adrenal, kidney, and aortic cholesterol were run in the case of rabbit 1 to check the methods used against the incorporation of random activity. This animal's body water had been labeled for only one day and it was known that after this short a time the specific activity of the cholesterol in these three sites would not be sufficient for measurement by the technic employed unless some form of label contamination occurred. In no case was there sufficient activity to be measured.

The 7 animals were killed serially; the specific activities of cholesterol found in the various organs as well as the average body water specific activities are summarized in table 1.

The half times for liver cholesterol in the animals examined varied widely, roughly from 10 to 20 days. The serum cholesterol specific activity approximated that of the liver cholesterol in all animals examined, suggesting a relatively rapid exchange of cholesterol between hepatic and serum cholesterol pools, since the liver undoubtedly supplies the bulk of the in vivo synthesized cholesterol of the serum.

Turning attention to the aorta it would seem possible to make some approximation of the turnover rate of aortic cholesterol from the data at hand. The following assumptions will be made: (1) that the aortic and serum cholesterol in the normal rabbit for the purpose of this calculation can be treated as single metabolic pools; (2) that cholesterol returning to the serum from the aorta will be in such small amounts as not to alter appreciably the specific activity of the serum cholesterol; and (3) that the turnover of aortic cholesterol uses the serum cholesterol as its major donor pool. It has been demonstrated in this laboratory in normal rabbits fed labeled cholesterol that both the serum and aortic cholesterol becomes labeled. Cholesterol synthesized within aortic tissue is neglected in this calculation. Then the turnover rate of aortic cholesterol can be estimated by solution of the equation $dN/dt = kN_1 - kN$* where N is the specific activity of the aortic cholesterol and N_1 equals the specific activity

---

* $dN/dt = kC(1 - e^{-k_1 t}) - kN$

$$N = C\left[1 + \frac{k_1 e^{-kt} - k e^{-k_1 t}}{k - k_1}\right]$$

of the serum cholesterol. N_1 will increase according to the usual growth of activity curve [$N_1 = C(1 - e^{-k_1 t})$] and the equilibrium value C has been shown to be half the specific activity of the body water.[2] The growth constants for specific activity of serum and aortic cholesterol are k_1 and k respectively.

Solving for k for rabbits 4, 5, and 6, one gets $\sim$0.29, $\sim$0.10 and $\sim$0.57 respectively, or half times of 2.4, 6.9, and 1.2 days for aortic cholesterol in normal rabbits. Whether or not it is permissible to neglect cholesterol synthesized within the aorta must await further study.

TABLE 1.—*Specific Activities of Tissue Cholesterol in Normal Rabbits Maintained with a Tritium-Labeled Body Water for Various Time Periods*

	Rabbit Number						
	1	2	3	4	5	6	7
Period of Experiment (*days*)	1	2	7	11	15	20	39
Initial wt. (*Gm.*)	4475	4300	4440	5775	3840	4440	3410
Sex	M	F	M	F	M	F	M
Initial T_2O Injection (*cc.*)	5.0	7.0	7.0	9.0	6.0	7.0	7.0
Daily Injection (*cc.*)	1.0	1.2	1.2	1.5	1.1	1.2	1.0
Tissue							
Liver	0.12	0.45	1.03	0.89	2.25	1.67	2.62
Adrenal			1.08	0.85	2.33	1.24	2.71
Serum			1.00	0.98	2.32	1.80	2.65
Lung				1.12	1.97	1.19	2.47
Brain				0.10	0.20	lost	0.37
Kidney				0.63	1.35	0.99	2.27
Heart				0.94	1.79	1.82	2.37
Aorta				0.69	1.02	1.68	1.92
Jejunum				1.94	2.90	lost	2.76
St. Muscle				0.51	0.82	0.73	1.81
Ovary				1.38		1.59	
Testicle			0.98		3.40		2.40
Average Body Water specific activity	3.38	6.32	5.82	6.60	7.40	6.86	5.63

PART 2

It seemed desirable to actually observe the deposition of fed cholesterol in the atheromas of the hypercholesterolemic rabbit although the circumstantial evidence indicated that such occurred. The general plan was to feed 2 rabbits tracer cholesterol for sufficient time to allow atheromas to develop and then to examine the aortic cholesterol for activity. Concurrently 2 other rabbits were to be fed unlabeled cholesterol for a similar period while their body water was maintained labeled with tritium at a constant level. In the first 2 animals exogenous cholesterol could be identified; in the second 2 endogenous cholesterol would be labeled. It was felt that a comparison of aortic cholesterol specific activities in the 4 animals at the end of the experiment would give some idea of what fraction of the cholesterol in the atherosclerotic aorta is of endogenous and what fraction of exogenous origin. In order to conserve tracer cholesterol it was deemed advisable to select animals which had been "primed" with unlabeled cholesterol. So the 2 to be given tritium-labeled cholesterol were first fed unlabeled cholesterol until they developed an elevated serum cholesterol and then the tracer studies were begun. In similar fashion the animals which were to have their body waters labeled were also allowed to develop an elevated serum cholesterol before tracer studies were started. It was known from past experience that most of the aortic deposits occurred after the hypercholesterolemia had developed.

At the end of the experiment the specific activities of cholesterol in various tissues, including aorta, were determined. A summary of the findings is recorded in tables 2 and 3.

These tables also include the feeding history of each animal and some descriptive information.

Rabbits 8 and 9 received an initial dose of labeled cholesterol of rather high specific activity and then the serum cholesterol was examined at intervals for activity. In rabbit 8 the specific activities of serum cholesterol were found to be: 22 hours after the cholesterol ingestion, 4.48; 48 hours, 7.86; and 72 hours, 8.57. In rabbit 9 the specific activity of serum cholesterol 48 hours after the initial feeding was found to be 4.28. The dosages of labeled cholesterol administered to each rabbit are indicated in the two tables. The administered cholesterol was dissolved in Wesson oil (0.5 Gm. cholesterol per 5 cc. Wesson oil) unless otherwise indicated. The initial tritium cholesterol doses were given by stomach tube;

TABLE 2.—*Cholesterol Specific Activities of Various Tissues in 2 Hypercholesterolemic Rabbits after Prolonged Tracer Cholesterol Feeding.*

	Rabbit Number	
	8	9
Sex	Female	Female
Initial wt.	5510 Gm.	5610 Gm.
Prep. feedings of unlabeled cholesterol	3.0 Gm. (not in oil) per wk. for 14 wks.	3.0 Gm. (not in oil) per wk. for 14 wks.; then 0.5 Gm. (in oil) per day for 30 days.
Serum cholesterol at start of labeling (*mg.%*)	1277	1250
Initial tracer cholesterol dosage	0.16 Gm. (s.a.* = 3.67×10^2)	0.12 Gm. (s.a. = 3.67×10^2)
Maintenance tracer cholesterol dosage	16 Gm. in 31 days (s.a. = 5.92)	23 Gm. in 39 days (s.a. = 3.32)
Period of tracer feeding	31 days	39 days
Final weight	5490 Gm.	5600 Gm.
Final serum cholesterol (*mg.%*)	1326	1140
Atheromas found	3+ (aorta contained 6.0 mg. of chol.)	3+ (aorta contained 4.0 mg. of chol.)
Tissue	**Specific Activity**	**Specific Activity**
Serum	3.83	3.05
Brain	0.14	0.08
Aorta	3.45	2.64
Lung	3.06	2.98
Liver	3.68	2.62
Kidney	3.10	2.66
Adrenal	2.99	2.46
Ovary	1.47	0.68
Jejunum	1.34	1.33

* s.a. = Specific Activity

TABLE 3.—*Cholesterol Specific Activities of Various Tissues in 2 Hypercholesterolemic Rabbits with Tritium-Labeled Body Water after Prolonged Cholesterol Feeding.*

	Rabbit Number	
	10	11
Sex	Male	Female
Initial wt.	5410 Gm.	5264 Gm.
Prep. cholesterol feedings before labeling	3.0 Gm. (not in oil) per wk. for 14 wks.; then 0.5 Gm. (in oil) per day for 30 days	3.0 Gm. (not in oil) per wk. for 7 wks.; then 0.5 Gm. (in oil) per day for 45 days.
Serum chol. at start of labeling (*mg. %*)	1250	458
Period during which body water was labeled	32 days	30 days
Unlabeled chol. fed during this period	16 Gm.	18 Gm.
Average body water s.a.	5.60	6.48
Final wt. (*Gm.*)	4770	5150
Final serum chol. (*mg.%*)	760	490
Atheromas found	4+ (aorta contained 11.5 mg. of chol.)	1+ (aorta contained 1.8 mg. of chol.)
Tissue	**Specific Activity**	**Specific Activity**
Brain	0.28	0.18
Aorta	0.22	0.33
Lung	0.34	0.35
Liver	0.27	0.29
Kidney	0.32	0.24
Adrenal	0.20	0.13
Gonads	Testes 0.65	Ovary 1.13
Jejunum	0.58	0.77
Serum	0.38	0.33

following doses were given in oil poured over the rabbit's food.

A comparison of the values of cholesterol specific activity in the 4 animals indicates clearly that most of the cholesterol in the tissue deposits of cholesterol fed rabbits, including aortic atheromas, are of exogenous origin. In rabbits 8 and 9 the specific activity of tissue cholesterol has begun to approach the specific activity of those fed labeled cholesterol even though the rabbits were "hypercholesterolemic" before tracer feedings were begun. Considering rabbits 10 and 11, we know from Part 1 that the synthesis rate of cholesterol in the normal animal is sufficiently rapid that in animals with a labeled body water the specific activity of the tissue cholesterol after 30 days approaches one-half the body water value (see rabbit 7). The specific activities of tissue cholesterol in rabbits 10 and 11 are only a very small fraction of this saturation value. The results here are again consistent with the absorption of large amounts of exogenous cholesterol.

TABLE 4.—*Summary of Findings in a Normal Rabbit Fed Tracer Cholesterol and Killed in Seven Days.*

Rabbit 12. *Normal Rabbit*
Feeding history: Purina Rabbit Chow
Initial weight: 2640 Gm.; Sex: male
Initial serum chol.: Free—3.1 mg.%; Total—24.8 mg.%
Tracer chol. given: 0.17 Gm. (s.a.=1.42×10^2) in 10 cc. Wesson oil
Period of experiment: 7 days

Cholesterol Source	Specific Activity	Per cent of given label in total serum or total organ cholesterol
1st day serum	16.0	
2nd day serum	18.9	≃2.6%
3rd day serum	13.3	
4th day serum	7.80	
5th day serum	6.49	
6th day serum	3.91	
7th day serum	4.01	≃0.5%
Liver	3.55	2.7%
Adrenal	3.94	0.2%
Kidney	3.06	0.8%
Lung	4.32	0.9%

It is noteworthy that cholesterol synthesis continues in the rabbit even in the face of massive cholesterol dosage by mouth, and a small amount of endogenous cholesterol is also to be found in the atheromatous aorta. Whether the rate of cholesterol synthesis is depressed by oral cholesterol feedings cannot be answered from the presented data, for the turnover rates of cholesterol in the tissues of hypercholesterolemic rabbits are not known. More work must be done to establish what dependence, if any, the in vivo cholesterol synthesis rate has on the amount of ingested cholesterol.

PART 3

To obtain some information on the fate of oral cholesterol, 5 rabbits were selected and each was fed by stomach tube a single dose of tritium-labeled cholesterol dissolved in warm Wesson oil. Then the specific activity of serum cholesterol was determined periodically in each case, and at death the specific activities of the cholesterol in the liver, adrenal glands, kidneys, and lungs were measured. The 5 animals of the experiment consisted of 1 normal animal, 3 hypercholesterolemic animals, and 1 "resistant" animal. The "resistant" animal had received cholesterol feedings for a total of 171 days. The total serum cholesterol had risen to only 222 mg. per cent. The ultracentrifugal pattern according to the technic of Gofman and associates[1] showed only a slight trace of "abnormal" molecules in the S_f 10–20 class. No atheromas were found in the aorta at death. The 3 hypercholesterolemic animals were strongly positive for S_f 10–30 class molecules and all had atheromas in the aorta at death. All animals received essentially the same oral dose of tritium-labeled cholesterol. The normal animal, one hypercholesterolemic rabbit, and the "resistant" animal were killed seven days after the tritium cholesterol feeding. The other 2 hypercholesterolemic animals were killed at 12 hours and 10 days respectively. A summary of the findings in these 5 animals is presented in tables 4, 5, 6, 7, and 8.

Only a relatively small portion of the label, fed as tritium-labeled cholesterol to these animals, was accounted for in the cholesterol of the serum, liver, adrenal glands, kidneys, and lungs at the end of each experiment. Less than 6 per cent of the original tritium dose was to

TABLE 5.—*Summary of Findings in a Hypercholesterolemic Rabbit Fed Tracer Cholesterol and Killed in Seven Days.*

Rabbit 13. *Hypercholesterolemic Rabbit*
Feeding history: 0.5 Gm. chol. in 5 cc. Wesson oil per day for 63 days
Initial weight: 4350 Gm.; Sex: female
Initial serum chol.: Free—189 mg.%; Total—651 mg.%
Tracer chol. given: 0.17 Gm. (s.a.=1.42 × 10^2) in 10 cc. Wesson Oil
Period of experiment: 7 days

Cholesterol Source	Specific Activity	Per cent of given label in total serum or total organ cholesterol
1st day serum	0.88	
2nd day serum	1.27	
3rd day serum	2.39	
4th day serum	3.42	≃11.5%
5th day serum	3.40	
6th day serum	2.11	
7th day serum	1.74	≃5.9%
Liver	1.30	12.5%
Adrenal	0.42	1.0%
Kidney	0.81	2.3%
Lung	1.24	4.4%

TABLE 6.—*Summary of Findings in a Hypercholesterolemic Rabbit Fed Tracer Cholesterol and Killed in Twelve Hours.*

Rabbit 14. *Hypercholesterolemic Rabbit*
Feeding history: 0.5 Gm. chol. in 5 cc. Wesson oil per day for 80 days
Initial weight: 4785 Gm.; Sex: female
Initial serum chol.: Free—312 mg.%; Total—1133 mg.%
Tracer chol. given: 0.18 Gm. (s.a.=1.42 × 10^2) in 10 cc. Wesson Oil
Period of experiment: 12 hours

Cholesterol Source	Specific Activity	Per cent of given label in total serum or total organ cholesterol
12 hour serum	0.38	≃0.5%
Liver	0.05	0.9%
Adrenal	0.005	0.01%
Kidney	0.10	0.05%
Lung	0.09	0.03%

be found seven days after the labeling feeding in the cholesterol of these sites in the normal (no. 12) and "resistant" (no. 14) animals. There was, however, more than four times this amount (~26 per cent) in the same sites in the hypercholesterolemic animal (13) also killed seven days after the initial tritium cholesterol feeding.

TABLE 7. *Summary of Findings in a "Resistant" Rabbit Fed Tracer Cholesterol and Killed in Seven Days.*

Rabbit 15. *"Resistant" Rabbit*
Feeding history: Crystalline cholesterol, 3 Gm. per week for 72 days, then 0.5 Gm. chol. in 5 cc. Wesson oil per day for 99 days.
Initial weight: 4450 Gm.; Sex: male
Initial serum chol.: Free—75 mg.%; Total—222 mg.%
Tracer chol. given: 0.17 Gm. (s.a.=1.42 × 10^2) in 10 cc. Wesson oil
Period of experiment: 7 days

Cholesterol Source	Serum Chol.		Spec. Activity		Per cent of given label in total serum or total organ cholesterol
	Free	Total	Free	Total	
1st day serum	83	222	0.75	0.83	
2nd day serum	79	220	1.40	1.85	
3rd day serum	62	197	1.72	2.94	
4th day serum	47	180	2.72	3.49	≃3.3%
5th day serum	40	171	2.55	2.98	
6th day serum	36	151	2.95	2.67	
7th day serum	28	121	1.86	1.90	≃1.3%
Liver				1.56	3.7%
Adrenal				0.60	0.1%
Lung				1.75	0.5%
Kidney				0.85	0.3%
Testicle				0.53	0.02%

TABLE 8. *Serum Cholesterol Specific Activities in a Hypercholesterolemic Rabbit Fed Tracer Cholesterol and Followed for Ten Days.*

Rabbit 16. *Hypercholesterolemic Rabbit*
Feeding history: 0.5 Gm. chol. in 5 cc. Wesson oil per day for 114 days
Initial weight: 3890 Gm.; Sex: female
Tracer chol. given: 0.2 Gm. (s.a.=1.42 × 10^2) in 10 cc. Wesson Oil
Period of experiment: 10 days

	Cholesterol Values		Specific Activity	
	Free	Total	Free	Total
1st day serum	266	793	0.61	1.22
2nd day serum	251	762	1.19	1.73
3rd day serum	247	866	1.42	1.93
4th day serum	246	780	1.48	1.34
5th day serum	243	805	1.24	1.08
6th day serum	258	780	1.03	1.06
8th day serum	219	780	0.83	0.81
10th day serum	168	740	0.65	0.38

In all animals examined, the specific activity of the serum cholesterol delayed reaching a maximum for a period of several days (two to five). See figures 1, 2, and 3. Rabbit 10 was

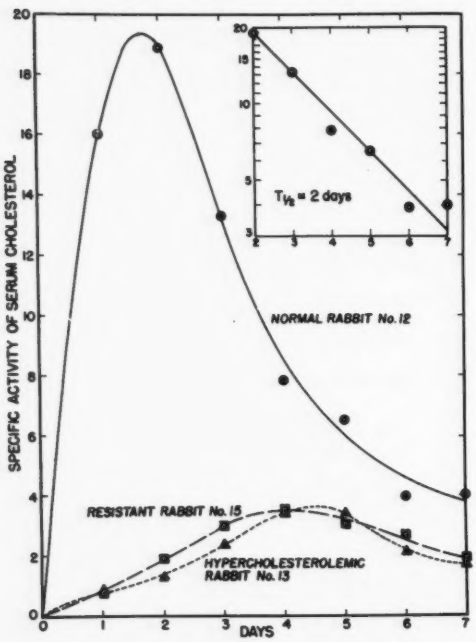


FIG. 1. Growth of specific activity of total serum cholesterol in 3 rabbits each fed a single dose of tritium-labeled cholesterol. (Inset: A semilogarithmic plot of the six descending values obtained in the case of the normal animal)

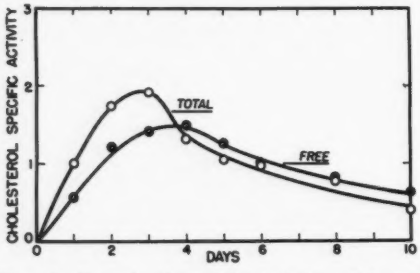


FIG. 2. Growth of specific activity of free and total serum cholesterol in rabbit 16 following the ingestion of a single dose of tritium-labeled cholesterol.

sacrificed 12 hours after feeding to see if this delay was due to storage of exogenous cholesterol in the liver prior to its liberation into the serum. No evidence that this occurs was obtained, for the specific activity of liver cho-

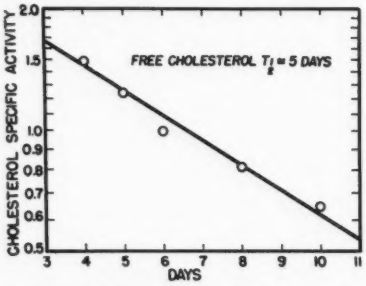


FIG. 3. A semilogarithmic plot of the descending values obtained for free cholesterol specific activity in rabbit 16.

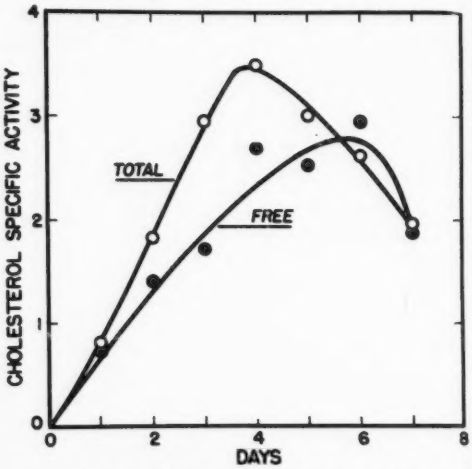


FIG. 4. Growth of specific activity of free and total serum cholesterol in rabbit 15 following the ingestion of a single dose of tritium-labeled cholesterol.

lesterol in this animal was found to be very low. It has been observed that such a fatty meal as the animals received when fed 10 cc. of Wesson oil causes anorexia for two or three days and a reduction in the bulk of the stools, and it is felt that the delayed maximum in the specific activity of the serum cholesterol is due to delayed absorption. The chow taken and the weight of the stools after the fatty cholesterol meal are given below for rabbit 13.

	Stool Wt.	*Chow Taken*
1st day	9.0 Gm.	none
2nd day	none	none
3rd day	none	20 Gm.
4th day	9.8 Gm.	35 Gm.
5th day	19.0 Gm.	55 Gm.
6th day	30.0 Gm.	60 Gm.
7th day	39.0 Gm.	75 Gm.

It was soon observed that the growth of activity in the free and total cholesterol fractions of the serum proceeded at different rates. The esterified cholesterol reached a higher maximum specific activity and reached it sooner. (See figs. 2 and 4.) The same sequence of events occurred in the "resistant" rabbit as did in the hypercholesterolemic animal examined.

If the descending values of the specific activities of free cholesterol of figure 2 are plotted on semilog paper (fig. 3), one obtains a half time of about five days for free cholesterol of the serum of the hypercholesterolemic animal studied. The more rapid rise and fall of specific activity in the esterified cholesterol of the serum

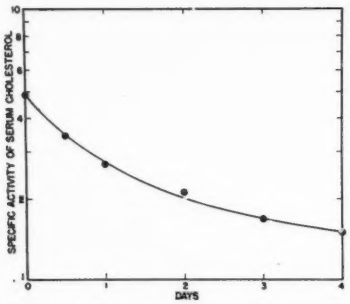


FIG. 5. The fall of total cholesterol specific activity in the serum of rabbit 17 following the intravenous injection of serum containing tritium-labeled cholesterol.

suggests that its turnover rate is more rapid than that of the free cholesterol. From figure 1 it can be seen that the half time of total serum cholesterol in the normal animal during the period of observation was about two days. Thus, if the half time in normal animals is about two days and that for the hypercholesterolemic one something less than five days, it is readily seen that the hypercholesterolemic animal was turning over in the serum many times the quantity of cholesterol handled by normal rabbit in serum per unit time.

Further data on the half time of serum cholesterol in a hypercholesterolemic rabbit was obtained in the following way. A donor animal with a total serum cholesterol level of 1980 mg. per cent was given tracer cholesterol dissolved in 10 cc. of Wesson oil. On the third day following administration of the cholesterol 25 cc. of blood were withdrawn and 11 cc. of serum therefrom administered intravenously to rabbit 17. This animal weighed 4450 Gm. and had a total serum cholesterol of 1512 mg. per cent. Eleven cc. of blood were withdrawn 30 minutes after the injection for the zero point and the subsequent results are recorded in figure 5.

There is evidence of more than one metabolic component. Whether or not these components are comprised of the various fractions as separated by Gofman[1] (S_f 8–10; S_f 17–20; etc.) must await further study.

The specific activity of the aortic cholesterol in the normal rabbit 12 was determined and found to be 1.83. The aorta of rabbit 16 was extensively involved with atheromatous deposits. The specific activity of the cholesterol here was found to be 0.16.

Much more work is needed on the turnover of cholesterol in the normal and atherosclerotic aorta. Work is at present under way to determine whether cholesterol derived from the different molecular groupings of the serum according to Gofman's technic has different turnover rates in aortic tissue.

SUMMARY

Radioactive hydrogen has been used to study certain aspects of cholesterol metabolism in the normal and hypercholesterolemic rabbit.

(1) Tritium-labeled cholesterol, when fed to normal and hypercholesterolemic rabbits, appears in the serum and eventually in all tissues examined. More of the tritium label can be accounted for in tissue and serum cholesterol in the hypercholesterolemic rabbit than in the normal animal seven days after a single tritium-labeled cholesterol feeding.

(2) Exogenous cholesterol forms the bulk of the cholesterol in the atheromatous deposits found in cholesterol fed rabbits.

(3) Following a single tritium cholesterol feeding the specific activity of esterified cholesterol of the serum rises to a higher level, and reaches a maximum faster than the free cholesterol. Indications are that esterified cholesterol of the serum is turning over faster than free cholesterol in the time period following cholesterol ingestion.

(4) The turnover of aortic cholesterol in 3 normal rabbits has been observed.

(5) Cholesterol synthesis continues in the rabbit even in the face of massive, prolonged cholesterol feeding by mouth.

ACKNOWLEDGMENT

The authors are indebted to Dr. John Gofman and Dr. Hardin Jones for their helpful criticism of this work, and to David Colman and Dean Graham for technical assistance.

REFERENCES

[1] GOFMAN, J. W., LINDGREN, F., ELLIOTT, H., MANTZ, W., HEWITT, J., STRISOWER, B., HERRING, V., AND LYON, T. P.: The role of lipids and lipoproteins in atherosclerosis. Science **111:** 166, 1950.
—, JONES, H. B., LINDGREN, F., LYON, T. P., ELLIOTT, H. A., AND STRISOWER, B.: Blood lipids and human atherosclerosis. Circulation **2:** 161, 1950.

[2] RITTENBERG, D., AND SCHOENHEIMER, R.: Deuterium as an indicator in the study of intermediary metabolism. XI. Further studies on the biological uptake of deuterium into organic substances, with special reference to fat and cholesterol formation. J. Biol. Chem. **121:** 235, 1937.

[3] BLOCH, K., AND RITTENBERG, D.: The preparation of deuterio cholesterol. J. Biol. Chem. **149:** 505, 1943.

[4] KRITCHEVSKY, D., BIGGS, M. W., AND FREEMAN, N. K.: Preparation of Tritiated Cholesterol. Berkeley, Univ. of Calif. Radiation Lab. Publication #644, 1950.

[5] FUKUSHIMA, D. K., AND GALLAGHER, T. F.: Platinum catalyzed hydrogen-deuterium exchange with steroids. Federation Proc. **9:** 174, 1950.

[6] SOBEL, A. E., AND MAYER, A. M.: Improvements in the Schoenheimer-Sperry method for the determination of free cholesterol. J. Biol. Chem. **157:** 255, 1945.

Hypercholesteremia and Atheromatosis in Chicks on a Restricted Diet Containing Cholesterol

By S. Rodbard, Ph.D., C. Bolene, M.S., and L. N. Katz, M.D.

Restriction of dietary intake even to the point of emaciation gave no protection against atheromatosis or hypercholesteremia in chicks on a diet supplemented with cholesterol. These results show that there is no necessary relationship between the amount of body fat and atherosclerosis.

EXCESS caloric intake and obesity have long been implicated in the development of atherosclerosis. This concept has been fostered by the common findings of an increased incidence of aortic and coronary atheromatosis in necropsies on overweight individuals.[1-4] Recent experiences arising in the backwash of the two great wars have been interpreted as strengthening this belief since the incidence of atherosclerosis was low in famine areas and concentration camps[5] but continued high in well fed troops.[2] The apparent freedom of the arterial tree from atheromatosis in starvation is illustrated by the fact that only a single reference to arterial pathology is given in the extensive review on human starvation by Keys and his co-workers.[5] However, there is considerable evidence which indicates that atherosclerosis may be closely associated with the composition of the diet rather than its amount. Thus atherosclerosis is uncommon among well fed Chinese[6] and Okinawans.[7] The presumed correlation between obesity and atheromatosis may therefore be challenged.

The finding that in some populations lavish caloric intake is correlated with atherosclerosis may not be dependent upon overfeeding as such. Instead it may be a function of a superfluity of some specific constituents of the diet, which incidentally are taken in excess by individuals on a luxus food intake. Support for this thesis has recently come in statistical analyses of the mortality experiences in the Scandinavian countries.[8, 9] In Denmark[8] and in Norway[9] a marked drop in mortality from circulatory diseases occurred during the German occupation. This was particularly notable in urban areas; it existed to a lesser degree in the rural districts where cholesterol-rich foods were still available in limited quantities.

The increasing evidence for the atherogenic role of cholesterol makes it necessary to assay the relative effects of excess calories and excess cholesterol intake in the production of arterial lesions.

A subsidiary problem concerns the possible utilization of excess dietary cholesterol for nutritional purposes in conditions of caloric privation. If this occurred, it might be expected that starved animals on such a cholesterol enriched diet would utilize the excess cholesterol and thus reduce the tendency to atheromatosis. It was therefore decided to study the tendency to hypercholesteremia and atheromatosis in animals on a restricted food intake high in cholesterol.

Methods and Results

To study these questions, 40 chicks, divided into three groups, were given a restricted diet enriched with either 8 per cent, 2 per cent or 0.25 per cent cholesterol. Control groups of chicks totaling 10 birds, received the same

From the Cardiovascular Department, Medical Research Institute, Michael Reese Hospital, Chicago, Ill. The department is supported in part by the Michael Reese Research Foundation.

Aided by a grant from the National Heart Institute (H 626).

Presented in preliminary form at the meeting of the American Association for the Study of Arteriosclerosis in November, 1949.

During the course of this study, one of us (C. B.) was Deborah V. Dauber Memorial Research Assistant.

diets ad libitum. The amount of cholesterol mash given to the animals on the restricted diet was about 60 per cent of that taken by the control groups. Six chicks received normal mash diets.

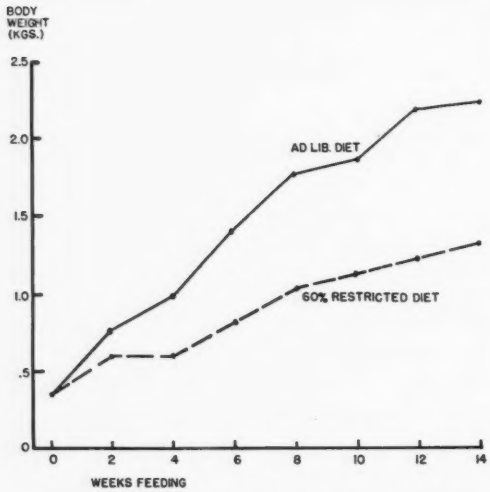


FIG. 1. Weight gains in chicks on ad libitum and restricted diets containing 0.25 per cent cholesterol and 5 per cent oil.

All these animals were carried until incidental death in the course of the experiment or until they were sacrificed at the end of 5, 10 or 15 weeks on the special diet.

The average plasma cholesterol increased from about 100 mg. per 100 cc. at the beginning of the experiment to 300 mg. per 100 cc. in one week of feeding. This approximate level was maintained, being 450 at five weeks, 400 at 10 weeks and 300 at 15 weeks.

One of 7 animals autopsied during the fifth week of feeding showed mild (grade 1) thoracic atheromatosis. Four of 9 animals autopsied at the tenth week had lesions graded as mild to severe ($\frac{3}{4}$ to $3\frac{1}{2}$). Four of the remaining 6 animals which were autopsied at the end of the fifteenth week had lesions varying from $\frac{1}{2}$ to 1. Three of these 6 animals were found at autopsy to be females. It was in 2 of these that no lesions were found. In the third, mild thoracic atherosclerosis graded as $\frac{1}{2}$ was observed.

Despite the restricted food intake, and the evident emaciation of the animals, atheromatosis was seen in 9 of 22 animals. This showed that atherogenesis may occur despite failure to gain weight at a normal rate.

TABLE 1.—*Plasma Cholesterol Levels and Atheromatosis of Chicks on Full and Restricted Diets*

	Weeks on diet	No. of chicks	Percentage with thoracic lesions	Average grade of thoracic lesions. Birds with lesions	Index‡	Plasma Cholesterol level mg. per 100 cc.
$\frac{1}{4}$ CO*	25	5	0	0.0	0.0	130
$\frac{1}{4}$ CO restricted diet	18–24	6	50	1.4	0.7	143
2 C†	15	5	80	1.8	1.4	550
2 C restricted diet	15	12	83	1.5	1.2	619

* $\frac{1}{4}$ CO = 0.25% cholesterol supplement with 5% oil
† 2 C = 2% cholesterol supplement
‡ Index = average grade of thoracic lesions of all birds

The lesions discussed in this study are those occurring in the thoracic aorta of birds on cholesterol enriched diets. The grading varies from 0 to 4 on the basis of size, number and elevation of the atheromatous plaques. The grading was done without knowledge of the group to which the individual bird belonged in order to add objectivity to the data.

A. Diets Containing 8 Per Cent Cholesterol. Twenty-two chicks placed in individual cages were given a limited mash diet enriched with 8 per cent cholesterol for periods up to 15 weeks.

It was clear that these animals did not utilize the excess cholesterol for energy purposes even in the face of obvious caloric privation. Instead, cholesterol was deposited in the blood vessels and various organs, particularly in the liver. These results made it appear of value to repeat the experiment with lower cholesterol dosages so that the effect of diet limitation on cholesteremia and atheromatosis could be assayed in birds on less abnormal diets.

B. Diets Containing 2 Per Cent Cholesterol. Seventeen animals were given a normal mash

diet until the age of 5 weeks. At this time they were divided into two groups and placed on the cholesterol diets. One group of 5 animals received an unstinted mash diet containing 2 per cent cholesterol. A comparable group of 12 animals received the same diet in amounts approximately two-thirds of the unrestricted group. The weight gain of the chicks on the restricted diet averaged about two-thirds of that of the chicks on the free regimen.

It can be seen from table 1 that the animals on the 2 per cent cholesterol limited diet had approximately the same level of plasma cholesterol (600 mg. per 100 cc.) and incidence and degree of atheromatosis as did their controls receiving the same mash in unlimited quantities. This was so despite the fact that the hungry birds received a notably reduced amount of cholesterol per bird. The reduced feed intake was evident in the retarded growth rate of these animals, in the diminished comb size, in the absence of gross body fat at autopsy and in the reduced testicular size.

C. Diets Containing 0.25 Per Cent Cholesterol. A similar pattern was seen in 6 chicks receiving 0.25 per cent cholesterol enriched diet in limited (two-thirds of control) amounts. At the end of 15 weeks of feeding, 3 of the 6 birds on the restricted diet had lesions with an average grade of 1.4, while none of the 5 birds on the full diet had lesions. The plasma cholesterol levels were similar in the two groups averaging about 140 mg per 100 cc. (table 1). Other findings such as weight (fig. 1), body fat, testicular development and comb size were similar to that seen in the groups on 2 per cent cholesterol mash.

Six control animals receiving an unlimited normal mash diet without the cholesterol supplement for periods up to 36 weeks were found to have plasma cholesterols averaging about 100 mg. per 100 cc. and no thoracic atheromatosis.

Discussion

The limited food intake clearly retarded the rate of growth of the chicks on this regime. Thus the chicks on the two-thirds diet gained weight at a rate approximately two-thirds that of the animals on the full diet. The retarded weight gain was evident in the smaller size of the birds. The influence of underfeeding on endocrine balance was evident throughout the experiment in the diminished comb and wattles development. There was little or no gross body fat. These hungry animals were more irritable than their well fed controls, pecking at once at anything that passed in front of their cages. When the daily allotment of food was placed in their trays, it was quickly eaten, leaving the food bins empty for most of the day. By contrast, the chicks with an abundant food supply ate continuously, although leisurely, throughout the day. Yet these well fed chicks consumed only one and one-half times as much food in the entire day as did the starved animals in an hour or so.

The cholesterol intake of the chicks in the 0.25 and 2 per cent cholesterol groups on the limited diet was approximately two-thirds that of their controls. However, the cholesterol taken per Kg. of chick was about the same for the two groups, inasmuch as the starved chicks were only two-thirds the weight of the well fed animals.

The plasma cholesterol levels were similar in the limited and in the well fed groups. The effect of exogenous cholesterol, all other conditions being equal, would therefore appear to depend on the ratio of cholesterol intake per Kg. of body weight. This relation is curvilinear, higher concentrations of cholesterol giving less effect per unit of this compound.[10]

Chronic malnutrition is known to result in a general lowering in the activity of the pituitary gland and through this organ, a reduced activity of the endocrine system as a whole. This effect was apparent in the reduced comb and wattle size of the malnourished animals as compared with the well-fed controls. The size of the comb is known to reflect the activity of the testes in particular and to some extent the adrenal cortical activity. In conformity with this, the testicles were found at autopsy to be much smaller in the starved chicks. This apparent reduced activity of the pituitary and the endocrine system as a whole either had no notable effect on the hypercholesteremia or atheromatosis, or its effect was neutralized.

The animals on a restricted diet also demonstrated a decreased resistance to cold as shown by the fact that some of them succumbed to cold when the room temperature fell during

the night, while well fed chicks in neighboring cages did not. This effect may have been due to the lack of subcutaneous fat insulation against cold.

Formulation

The fact that the plasma cholesterol increases despite the inadequate caloric intake demonstrates that there is a limit to the extent to which ingested cholesterol can be degraded for energy purposes.

Our results clearly demonstrate that atherosclerosis can develop in chicks on a very strictly limited diet. Thus the belief that obesity is necessarily related to atherogenesis is seriously challenged. Our data suggest that the degree of atherosclerosis may even be somewhat greater in the chicks on the restricted diet containing cholesterol. It becomes apparent that the atheromatosis is due to ingested cholesterol rather than to the amount of food taken. Firstbrook has recently challenged this interpretation on the basis of similar studies on rabbits.[11] Utilizing multiple correlative technics, he reported that cholesterol-fed rabbits receiving either ad libitum or limited diets showed a high net correlation between relative weight gain and the *severity* of experimental lesions. Statistical handling of the arbitrary judgment of the grades of atheromatous lesions is open to question since the significance of a change from grade 0 to 1 is much greater than, for example, from 3 to 4. Analysis of his published data from this point of view reveals that the *incidence* of atheromatosis is high in the rabbits which did not gain, or even lost weight on the experimental cholesterol diets. Firstbrook's data may therefore be considered as supporting our thesis that cholesterol-induced atherogenesis proceeds unchecked even at reduced levels of caloric intake.

The relation between obesity and atherosclerosis apparently depends on the fact that caloric excess in Western nations often signifies an increased cholesterol intake. In other nations, ungoverned caloric intake with its consequent obesity may take place with little cholesterol in the diet and with a low incidence of arteriosclerosis. Therefore the presumed correlation between overweight and atherosclerosis is probably coincidental.

Summary

The effect of a limited intake of a cholesterol enriched diet on the tendency to hypercholesteremia and atherogenesis was studied in chicks. Limitation of dietary intake to two-thirds of that normally taken did not protect against the development of hypercholesteremia and atherosclerosis in these malnourished animals.

The tendency to atherosclerosis and hypercholesteremia is correlated with the cholesterol ingested per Kg. of body weight of the animal. Atherosclerosis is thus shown to occur in the starved animals and in the absence of hyperphagia or obesity.

Cholesterol apparently can be utilized to only a limited degree for energy purpose even in the face of severely restricted caloric intake.

Acknowledgments

The authors are indebted to Miss Marilyn Dudley, B.S., for cholesterol determinations and to Mr. Philip Johnson for technical assistance in the dietary management of these experiments.

REFERENCES

[1] Dublin, L. I., and Marks, H. H.: The influence of weight on certain causes of death. Human Biol. **2:** 159, 1930.

[2] French, A. J., and Dock, W.: Fatal coronary arteriosclerosis in young soldiers. J. A. M. A. **124:** 1233, 1944.

[3] Wilens, S. L.: Bearing of nutritional state on atherosclerosis. Arch. Int. Med. **79:** 129, 1947.

[4] Wilens, S. L.: The resorption of arterial atheromatous deposits in wasting disease. Am. J. Path. **23:** 793, 1947.

[5] Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H. L.: The Biology of Human Starvation. Minneapolis, Univ. Minnesota, 1950.

[6] Oppenheim, F.: Review of 100 autopsies of Shanghai Chinese. Chinese M. J. **39:** 1067, 1925.

[7] Steiner, P. E.: Necropsies on Okinawans. Arch. Path. **42:** 359, 1946.

[8] Malmros, H.: The relation of nutrition to health. Acta med. Scandinav. Supp. **246:** 137, 1950.

[9] Strøm, A., and Adelsten Jensen, R.: Mortality from circulatory diseases in Norway. Lancet **1:** 126, 1951.

[10] Horlick, L., and Katz, L. N.: The relationship of atheromatosis development in the chicken to the amount of cholesterol added to the diet. Am. Heart J. **38:** 336, 1949.

[11] Firstbrook, J. B.: The effect of changes in body weight on atherosclerosis in the rabbit. Science **111:** 31, 1950.

Respiratory Complications from Tetraethylammonium Ion

Report of Two Deaths

By GEORGE A. MCLEMORE, JR., M.D., HAROLD D. GREEN, M.D., AND THOMAS N. LIDE, M.D.

Ill effects of tetraethylammonium ion reported in the current literature are reviewed. Two deaths are reported which are considered to be attributable to this substance; one resulted from a myocardial infarction, the other was due to respiratory failure. Four dogs were given progressively increasing doses of tetraethylammonium ion and all ultimately died of respiratory failure.

THE tetraethylammonium ion has been used quite extensively since 1946, when Acheson and Moe[1, 2] demonstrated its action as a blocking agent, effective at both sympathetic and parasympathetic ganglions. Earlier, Burn and Dale[3] and Hunt,[4, 5] had described the actions of certain quaternary ammonium bases which included the tetraethylammonium ion.* This drug has been used therapeutically with varying success in a variety of disorders, including thromboangiitis obliterans and arteriosclerosis obliterans,[6-9] coronary heart disease,[10] hypertension and toxemia of pregnancy,[11, 12] thrombophlebitis, Raynaud's phenomenon, trench foot and immersion foot,[13] peptic ulcer,[14, 15] poliomyelitis,[16] herpes zoster and intercostal neuralgia.[17] Diagnostically, tetraethylammonium chloride has been employed in the evaluation of many acrovascular conditions,[18-20] in the selection of patients with neurogenic hypertension for sympathectomy,[21] and in the roentgen study of the small bowel.[22]

Reports of serious reactions from the use of tetraethylammonium chloride have not been numerous, but unpleasant side effects have somewhat limited its usefulness,[23] as has the necessity for parenteral administration due to apparent lack of absorption when given by mouth. Another disadvantage has been the relatively short duration of action due to rapid excretion by the kidneys. Fifty per cent appears in the urine in 30 minutes when given intravenously, and 50 per cent in four hours when given intramuscularly.[24] Subcutaneous injection causes considerable irritation and tenderness, which may last for several hours, at the site of injection. For this reason this method of administration is not recommended. Intramuscular injection also produces some tenderness, mild burning and occasional muscular fasciculation, but the effect is more prolonged. The latter route of administration is probably the safest as regards systemic reactions in doses recommended not to exceed 20 mg. per Kg. of body weight. Rapid intravenous injection of tetraethylammonium chloride has been used by many reporters and is generally employed as recommended by Parke, Davis and Company in amounts not exceeding 7 mg. per Kg. of a 10 per cent solution.

The effects of rapid intravenous administration usually appear immediately and include metallic taste, a cold feeling, weakness, drop in arterial blood pressure, rise in pulse rate, ablation of sweating, lightheadedness, and difficulty of muscle movement without impairment of the deep tendon reflexes. Temporary loss of ocular accommodation usually occurs and may last several hours. Dyspnea and hyperventilation, similar to hysterical hyperventilation, together with retention and drying of bronchial secre-

From the Department of Physiology and Pharmacology, the Department of Internal Medicine, and the Department of Pathology, Bowman Gray School of Medicine of Wake Forest College, and The North Carolina Baptist Hospital, Winston-Salem, N. C.

This project was supported by cardiovascular Research Grant H-487 and cardiovascular Teaching Grant HT-344 from the National Heart Institute, U. S. Public Health Service.

* Etamon chloride kindly supplied by Parke, Davis and Company, Detroit, Mich.

tions, may occur, especially in female subjects, when large doses are administered.[25]

It is the purpose of this paper to review some of the more serious complications that have been reported from the use of tetraethylammonium chloride, and to report 2 cases of respiratory cessation and death. We will also give a preliminary report of work begun with dogs on some toxicologic aspects of tetraethylammonium chloride.

Review of Previous Reports of Toxicity of Tetraethylammonium Chloride

Friedlich and Stansbury[26] reported an instance of a severe reaction to the intravenous administration of Etamon in an emotionally unstable 37 year old female patient. After receiving 230 mg. of tetraethylammonium chloride, she developed peripheral collapse, hyperventilation, gasping respirations and shivering. She required intensive supportive therapy in the form of plasma, adrenaline and whole blood over a two day period before recovery. Schwartz[27] reported the sudden death, without apparent cause, of a patient with rather severe bronchial asthma, following the use of tetraethylammonium bromide. Autopsy revealed generalized congestion and the possibility of ventricular fibrillation. In this case also the drug was administered intravenously, and the dose was 230 mg. The patient, a 63 year old white man, expired very suddenly; epinephrine and artificial respiration were not of benefit. Lasser and others[28] reported a case of advanced hypertensive cardiovascular disease who developed circulatory collapse. This patient was a 33 year old white woman with severe hypertensive cardiovascular disease whose disease had continued to progress despite sympathectomy. She was given 500 mg. of tetraethylammonium chloride intravenously over a five minute period. Following the administration of tetraethylammonium chloride she developed peripheral collapse which was unsuccessfully treated with Coramine (nikethamide) and epinephrine. She died six hours later. Cyanosis and labored respirations were outstanding features, and at postmortem examination red cells, large macrophages, occasional polymorphonuclear leukocytes, and eosinophilic granular material were found in most alveoli. The other findings were those of advanced hypertensive cardiovascular disease with severe nephrosclerosis. Ham[29] reported a case of purpura occurring three days after the administration of tetraethylammonium chloride but no causal relationship was established.

Green and Ogle[18] have used tatraethylenmonium chloride in inducing a rise in skin temperature in peripheral vascular disease. In a series of 20 cases, they found few signs of toxicity in doses not exceeding 600 mg. given intravenously. Later, Green and others[19] reported a larger series of skin temperature studies employing tetraethylammonium chloride, and mentioned a case of an elderly white man who expired during a skin temperature study after receiving only 7.8 mg. per Kg. of tetraethylammonium chloride in a normal saline infusion. This patient is reported as case 1.

Ulrich and co-workers[30] gave tetraethylammonium bromide by slow intravenous infusion at a rate of 6 to 10 mg. per minute for four to eight hours. They found that elevated skin temperature could be maintained for six to eight hours in normotensive and hypertensive individuals by this method with few, if any, side reactions. From 12 mg. per minute up to 16 mg. per minute, the maximum amount used, moderate untoward effects were said to have occurred but they were not enumerated. The vehicle of infusion was not mentioned. The maximal rise in skin temperature was obtained with the largest amount, 16 mg., of tetraethylammonium bromide per minute.

Tetraethylammonium chloride has been used almost exclusively by intravenous infusion in normal saline by the group of workers on peripheral vascular diseases in the Department of Physiology and Pharmacology, and the Department of Internal Medicine at the Bowman Gray School of Medicine,[19] and it has been used in this manner since May, 1947, with generally good results in obtaining a rise in skin temperature and relief of symptoms in vasospastic conditions. Sixty patients with a variety of vascular disorders and 50 normal medical students have received doses of tetraethylammonium chloride ranging from 300 mg. to 1800 mg. in

the saline diluent over periods of 30 to 45 minutes without serious untoward effects. The above patients were generally given the drug for diagnostic purposes while recording skin temperatures. Twenty of these cases were then treated over a period of days in the same manner, by intravenous infusion with close watch for toxic signs and symptoms. Only 3 cases showed any untoward signs, namely drop in systolic blood pressure of 30 to 40 points, weakness, and occasional dyspnea.

In view of the fact that the rise of skin temperature was nearly proportional to the amount of tetraethylammonium chloride infused per minute, the total amount of this drug used for treatment was cautiously raised over an extended period of time. It was found that the desired increase in skin temperature was obtained with a dose of 20 mg. per Kg. of body weight, usually given at the rate of 40 to 50 mg. per minute. Mild transient ill effects occasionally occurred, but no serious result was noted until this latter dose had been used on a total of 9 patients. The ninth patient died during the course of treatment. A summary of the clinical course and postmortem findings in this patient are reported as case 2.

Case Reports

Case 1. D. V. C., a 78 year old white man entered the North Carolina Baptist Hospital, Winston-Salem, N. C., on Sept. 15, 1949, because of severe and sudden pain and coldness in his right leg. A purple hue to the right foot and ankle up to the midcalf had developed three days prior to admission. No history of chest pain, heart disease or hypertension was obtained. He had been nauseated, however, and vomited once on the day of admission.

On admission the temperature was 99 F., the pulse 84 beats per minute, respirations 18, and blood pressure, 150/90 in both arms. He was a fairly well-developed, elderly, slightly obese, white male in acute distress. His eyes revealed an arcus senilis and grade II arteriosclerotic changes in the fundi. The lungs were clear. The heart was normal in shape and size with a regular sinus rhythm, with sounds of good quality, but with an occasional premature systole. The distal portion of the right lower extremity was cool and purple in color below the midcalf. Pulsation could not be obtained in the popliteal, tibial or dorsalis pedis arteries of the right leg, and the right femoral pulsation was not as strong as the left.

Laboratory examination showed a normal urinalysis except for 20 white cells per high powered field and an occasional granular cast. The hemoglobin was 16 Gm., and the corrected sedimentation rate 10 mm. in one hour. The white blood cell count was 14,750, with a slight increase in the segmented polymorphonuclear leukocytes.

His hospital course was brief. Shortly after admission, he was placed in the constant temperature room at 20 C. where a skin temperature study was started, using tetraethylammonium chloride, 1000 mg. in 300 cc. of dextrose and saline. He vomited about one-half cupful of light brown material at the start of the study before administration of the tetraethylammonium chloride was begun but became quiet after this while the infusion was being carried out. Seventeen minutes after the infusion was started, his blood pressure dropped from 140/80 to 120/60; he again became nauseated but did not vomit. The infusion was stopped for five minutes whereupon his blood pressure returned to the original level; the pulse remained slow, 65 to 70 per minute. The infusion was then continued until 650 mg. of tetraethylammonium chloride had been given, when suddenly his blood pressure became unobtainable, his pulse rapid and weak, his respirations shallow and weak and he became very relaxed and did not respond. A total of 1 cc. of 1:1000 epinephrine, 0.5 cc. intravenously and 0.5 cc. intramuscularly, was not successful in reviving the patient, nor was artificial respiration. He was pronounced dead 40 minutes after the start of the skin temperature study.

Autopsy findings revealed a severe generalized arteriosclerosis, with occlusion of the anterior descending branch of the left coronary artery. The heart was slightly hypertrophied, weighing 400 Gm. Congestion was prominent throughout all organs, with edema and hemorrhage into the pulmonary alveoli, and petechial hemorrhages of the gastric mucosa. Fatty metamorphosis was moderate in the liver, but there was no lobular necrosis. The kidneys were small and scarred, with sclerosis of the arteries and arterioles, and glomerular and interstitial fibrosis.

Case 2. E. S. H. was admitted to the North Carolina Baptist Hospital, Winston-Salem, N. C., on Dec. 23, 1949. He was a 33 year old white grocer, veteran of the Battle of the Bulge, Dec. 1944. During 1944 he had suffered "trench foot" from long exposure to cold, wet weather and was hospitalized for several weeks of treatment. He was then sent back to the front and later discharged without disability. He was without symptoms for the following three years. In the winter of 1947 to 1948, his feet began to pain him. The pain was more severe in cold weather and after walking a short distance. The feet would become blue and cold and would usually swell. He had no difficulty in warm weather. He was admitted for treatment with tetraethylammonium chloride with a diagnosis of Raynaud's phenomenon

secondary to "trench foot." He gave a history of consuming about a quart of whiskey per day "for relief of the pain in his feet."

On admission the temperature was 98.8 F., pulse 112 per minute, respirations 18 per minute, weight 175 pounds and arterial blood pressure 146/104. He was a well developed, well nourished white man who was moderately nervous. Physical examination was generally unremarkable except for an upper denture and coldness and pallor of both feet. The latter was intensified and accompanied by severe pain when the feet were immersed in cold water. The feet became a blotchy purple upon removal from the cold water.

Laboratory findings were all within normal limits, with the exception of a corrected sedimentation rate of 23 mm. in one hour. The patient's prothrombin time was 14.5 seconds with a control of 15 seconds.

A skin temperature study, carried out the day of admission, showed a very good response of his feet and hands to 1580 mg. of tetraethylammonium chloride given in 250 cc. of saline over a 37 minute period. Since he experienced no apparent ill effect, he was started on a course of tetraethylammonium chloride therapy. Fifteen hundred mg. in 200 cc. of normal saline were given four times a day over a 30 minute period for a total of four days. Four hours usually elapsed between each infusion. Careful checks were made of blood pressure at 10 minute intervals throughout and following each infusion. At no time did either systolic or diastolic pressure drop more than 8 to 10 mm. Hg. His pulse was elevated much of the time during his hospital stay (100 to 126 per minute). He was given a total of 600 mg. of dicumarol over a five day period but he never displayed any signs of toxicity and his prothrombin time never exceeded 19 seconds with a control of 17 seconds.

On his sixth hospital day, Dec. 27, 1949, he was given the second infusion for that day, three and one-half hours after the previous infusion. Twenty minutes after the completion of this second infusion, which was given over a period of 35 minutes, he suddenly became excited, appeared to be in collapse and stopped breathing. Manual artificial respiration was begun immediately but was of little avail in that the patient's thoracic cage was apparently fixed in inspiration. Positive pressure artificial respiration was started 20 minutes later, but it also failed to correct the developing cyanosis. Adrenaline given intravenously was used in an attempt to maintain blood pressure, and 2 cc. of Coramine (nikethamide) were administered intravenously in an effort to stimulate respiration. Tracheotomy was performed in belief that there might be tracheal or laryngeal obstruction. Strong heart action was maintained for approximately 30 minutes but spontaneous respiration was never resumed. The blood pressure dropped gradually and the patient became intensely cyanotic. He was declared dead one hour and five minutes after completion of the infusion.

At autopsy there was generalized congestion of all organs, with petechial hemorrhages of the lungs, pleura, pericardium and mucosa of the gastrointestinal tract, particularly the ileum. Pulmonary edema with focal hemorrhages, and with acute bronchitis was prominent, but there was no pneumonic consolidation. The liver weighed 3000 Gm. and showed extreme fatty metamorphosis and congestion without demonstrable increase in fibrous tissue. There was extensive cloudy swelling and necrosis of the renal tubular epithelium. Cardiac hypertrophy, predominantly involving the left ventricles, was moderate (530 Gm.).

Discussion of Cases

The death of the 78 year old patient, case 1, was obviously due to the recent myocardial infarction which was not suspected ante mortem. His death was attributed to circulatory collapse. Respiratory difficulties were probably secondary.

The unexpected death of case 2 gave rise to much speculation as to the actual cause of the marked respiratory symptoms and fixation of the chest in inspiration. A total of sixteen previous infusions of the same amount had been administered over the same length of time for five days under close supervision, especially of blood pressure and pulse. He had been kept lying flat on his back for one hour after completion of each infusion. Though he had complained of blurred vision and slight dyspnea, no significant decline of blood pressure, no fever and no sign of collapse were ever noted. There were no signs or symptoms of pneumonia. The possibility of a correlation between the dicumarol therapy and the changes in the liver was considered; it was, however, felt to be unlikely in view of the failure of his prothrombin time to exceed 19 seconds, despite a total administration of 600 mg. over a five day period. The death may therefore have been due to depression of the respiratory center by the tetraethylammonium chloride. There were no signs of renal damage that might have caused retention of the tetraethylammonium chloride. Nevertheless it is possible that a chronic cumulative toxic effect of the tetraethylammonium chloride was responsible for the respiratory depression. It is possible that the fatty meta-

morphosis in the liver may have contributed in some unknown manner to the chronic toxicity of the tetraethylammonium chloride since all 4 of the dogs (described below) survived considerably larger doses of tetraethylammonium chloride than this patient received.

Studies on Animals

Gruhzit and others[31] studied the acute and chronic toxicity of tetraethylammonium chloride in mice, rats, rabbits and dogs. They reported little toxicity even after 2636 mg. per Kg. were given orally, but moderate signs were noted after 25 mg. per Kg. were given intramuscularly. These consisted of incoordination, ptosis of eyelids, mydriasis, and hyperemia of ocular, nasal and buccal membranes. As expected, these toxic signs were more marked with intravenous administration without diluent; at the dose which produced death in 50 per cent of the animals, the above signs were intensified with the addition of hyperpyrexia, spasticity, and respiratory and circulatory depression eventually leading to death over a period varying from immediately following administration to two and one-half hours after administration. No changes could be found in the blood studies, total nonprotein nitrogen, bromsulfalein test, total serum protein, albumin and globulin ratios, or in repeated urinalysis. The underlying histopathologic changes were those of severe congestion, stasis, edema, and anoxia.

We have carried out preliminary toxicity studies in 4 dogs. These dogs were given tetraethylammonium chloride in 100 cc. of normal saline over a 30 minute period four times a day. They received initial doses of 20 mg. per Kg. per infusion for two days. The dose was then increased by 5 mg. per Kg. every two days thereafter. The dogs died after receiving 24, 25, 41 and 49 infusions. The amounts of tetraethylammonium chloride which they were receiving per infusion just prior to death were 30, 35, 45 and 50 mg. per Kg. respectively. The respiratory complications in these animals largely simulated the effects noted in the second case report, namely, difficult irregular respirations, with respiratory cessation as the cause of death in each of the 4 dogs. Fixation of the thorax in inspiration was noted in 3 of the 4 animals, as was the case in case 2. The lethal dose was slightly higher than that reported by Gruhzit who used tetraethylammonium chloride undiluted, and who reported the minimum lethal dose to be 36 mg. per Kg.

The autopsy findings in all 4 dogs were similar, except in dog no. 2, in which an extensive confluent bronchopneumonia was found. The prominent and pertinent features of the autopsies were the edema and focal hemorrhage into the pulmonary alveoli and the extreme generalized congestion of the systemic and pulmonary blood vessels. Hyperemia of the gastrointestinal mucosa was present in each dog, the site of the change being predominantly the stomach and upper part of the small intestine. There was no necrosis of the hepatic cells, and there was but slight degeneration of the renal tubular epithelium; this was limited to the loops of Henle.

Reardon and others[32] described the effects of prostigmine in counteracting the toxic actions of tetraethylammonium chloride in dogs and man. The use of prostigmine could not be found in the cases described earlier in this article, nor in other reports reviewed. Prostigmine has been unsuccessful in preventing respiratory failure thus far in our studies on other dogs which will be reported in full at a later date.

Summary and Conclusions

A review of the more serious ill effects of tetraethylammonium chloride is presented. At least two deaths following its use have been reported. Two additional deaths following tetraethylammonium chloride are presented in this paper as case reports. Myocardial infarction with circulatory collapse was the probable cause of death in case 1. Case 2 had tolerated without incident 20 mg. per Kg. of tetraethylammonium chloride, intravenously, four times a day at four hour intervals for five days; each infusion had required at least 30 minutes. On the sixth day of treatment, the patient died at the conclusion of the second infusion for that day. This case showed at autopsy generalized congestion, pulmonary edema with focal hemorrhages and acute bronchitis, and fatty metamorphosis and congestion of the liver. Chronic

alcoholism was probably responsible for the changes in the liver. Death was due to respiratory failure.

Four dogs received tetraethylammonium chloride in an intravenous infusion over a 30 minute period four times a day. All 4 dogs died with respiratory failure. Three of the 4 dogs revealed cessation of respiration in the inspiratory phase. Postmortem pulmonary findings on all 4 dogs revealed edema and focal hemorrhage into the pulmonary alveoli, and generalized congestion including the lungs. The lethal doses in these 4 dogs ranged from 30 to 50 mg. per Kg. (average 40 mg. per Kg. per infusion).

REFERENCES

[1] Acheson, G. H., and Moe, G. K.: Some effects of tetraethylammonium on the mammalian heart. J. Pharmacol. & Exper. Therap. **84:** 189, 1945.

[2] —, and —: The action of tetraethylammonium on the mammalian circulation. J. Pharmacol. & Exper. Therap. **87:** 220, 1946.

[3] Burn, J. H., and Dale, H. H.: The action of certain quarternary ammonium bases. J. Pharmacol. & Exper. Therap. **6:** 417, 1915.

[4] Hunt, R., and Renshaw, R. R.: On some effects of arsonium, stibonium, phosphonium and sulfonium compounds on the autonomic nervous system. J. Pharmacol. & Exper. Therap. **25:** 315, 1925.

[5] —, and —: Some effects of quaternary ammonium compounds on the autonomic nervous system. J. Pharmacol. & Exper. Therap. **28:** 367, 1926.

[6] Moe, G. K., Rennick, B. R., Capo, L. R., and Marshall, M. R.: Tetraethylammonium as an aid in the study of cardiovascular reflexes. Am. J. Physiol. **157:** 158, 1949.

[7] Fisher, M. M.: Tetraethylammonium chloride in peripheral vascular disease and allied conditions; its uses and limitations. New York State J. Med. **49:** 1033, 1949.

[8] Coller, F. A., Campbell, K. N., Berry, R. E. L., Sutler, M. R., Lyons, R. H., and Moe, G. K.: Tetraethylammonium as an adjunct in the treatment of peripheral vascular disease and other painful states. Ann. Surg. **125:** 729, 1947.

[9] Berry, R. L., Campbell, K. N., Lyons, R. H., Moe, G. K., and Sutler, M. R.: The use of tetraethylammonium in peripheral vascular disease and causalgic states; new methods producing blockade of autonomic ganglia. Surgery **20:** 525, 1946.

[10] Christy, H. W.: Clinical evaluation of tetraethylammonium chloride in coronary heart disease. Am. J. Med. **6:** 668, 1949.

[11] Tamagna, J. G., and Poindexter, C. A.: A comparative evaluation of tetraethylammonium chloride and sodium amytal in patients with hypertensive cardiovascular disease. Am. J. M. Sc. **215:** 651, 1948.

[12] Brust, A. A., Assali, N. S., and Ferris, E. B.: The evaluation of neurogenic and humoral factors in blood pressure maintenance in normal and toxemic pregnancy using tetra-ethylammonium chloride. J. Lab. & Clin. Med. **33:** 1466, 1948.

[13] Ochsner, A., and DeBakey, M.: Peripheral vascular disease; critical survey of its conservative and radical treatment. Surg., Gynec. & Obst. **70:** 1058, 1940.

[14] Cayer, D. J., Little, J. M., and Yeagley, J.: Use of tetraethylammonium chloride in the treatment of patients with peptic ulcer. Gastroenterology **12:** 219, 1940.

[15] Little, J. M., Ogle, B. C., Yeagley, J., and Cayer, D. J.: Effect of tetraethylammonium in experimental gastric ulceration in the rat. Science **106:** 448, 1947.

[16] Lannon, J., and Braudo, J. L.: Muscle spasm in poliomyelitis. South African M. J. **23:** 30, 1949.

[17] Fisher, R. L., Zuckerman, M., and Sweeney, D. N., Jr.: Tetraethylammonium chloride in treatment of herpes zoster and intercostal neuralgia. Arch. Neurol. & Psychiat. **61:** 194, 1949.

[18] Green, H. D., and Ogle, B. C.: Use of vasodilator drugs and body warming in peripheral vascular disease. J. Appl. Physiol. **1:** 663, 1948.

[19] —, Perkins, W., and Abernethy, J.: Evaluation of the severity of organic occlusive disease and comparison of the effectiveness of various procedures in relaxing peripheral vasospasm. Severity evaluated by determining cutaneous blood flow in the extremities from records of cutaneous temperature during maximum vasodilation. Effectiveness of spinal anesthesia, intravenous tetraethyl ammonium ion (Etamon), intravenous benzylimidazoline (Priscoline) and application of heat to the torso. Circulation **1:** 1277, 1950.

[20] Lyons, R. H., Moe, G. K., Neligh, R. B., Hoobler, S. W., Campbell, K. N., Berry, R. L., and Rennick, B. R.: The effects of blockade of the autonomic ganglia in man with tetraethylammonium; preliminary observations on its clinical applications. Am. J. M. Sc. **213:** 315, 1947.

[21] Birchall, R., Taylor, R. D., Lowenstein, B. E., and Sage, J. H.: Clinical studies of pharmacologic effects of tetraethylammonium chloride in hypertensive persons made in attempt to select patients suitable for lumbodorsal sympathectomy and ganglionectomy. Am. J. M. Sc. **213:** 572, 1947.

[22] Haden, W. D., Jr., Perry, S. P., and Shallen-

BERGER, P. L.: The use of tetra-ethyl-ammonium as a diagnostic aid in roentgen study of the small bowel. The Guthrie Clin. Bull. **18:** 157, 1949.

[23] LINTON, R. R.: Peripheral vascular disease. New England J. Med. **240:** 645, 1949.

[24] RENNICK, B. R., MOE, G. K., LYONS, R. H., HOOBLER, S. W., AND NELIGH, R.: Absorption and renal excretion of tetraethylammonium ion. J. Pharmacol. & Exper. Therap. **91:** 210, 1947.

[25] Etamon Chloride, a New Ganglionic Blocking Agent. Detroit, Parke, Davis and Company, Mich.

[26] FRIEDLICH, A. L., JR., AND STANSBURY, J. B.: Severe reaction to tetraethylammonium chloride. New England J. Med. **238:** 629, 1948.

[27] SCHWARTZ, M.: Sudden death from tetraethylammonium bromide. Lancet **1:** 987, 1949.

[28] LASSER, R. P., ROSENTHAL, N., AND LOEWE, L.: Death following the use of tetraethylammonium chloride. J.A.M.A. **139:** 153, 1949.

[29] HAM, F. F.: Purpura following treatment with tetraethylammonium chloride. California Med. **69:** 279, 1948.

[30] ULRICH, C. W., PIERCE, J. D., JR., AND KOHLSTAEDT, K. G.: Administration of tetraethylammonium bromide by slow continuous intravenous infusion. Am. J. Med. **6:** 664, 1949.

[31] GRUHZIT, O. M., FISKEN, R. A., AND COOPER, B. J.: Tetraethylammonium chloride $[(C_2H_5)_4NCl]$. Acute and chronic toxicity in experimental animals. J. Pharmacol. & Exper. Therap. **92:** 103, 1948.

[32] REARDON, M. J., MARZONI, F. A., AND HENDRIX, J. P.: The effect of neostigmine (Prostigmine) on the actions of tetraethylammonium (Etamon) in dogs and man. Federation Proc. **6:** 364, 1947.

Congestive Heart Failure
Variations in Electrolyte Metabolism with Salt Restriction and Mercurial Diuretics

By Richard J. Stock, M.D., Gilbert H. Mudge, M.D., and Miriam J. Nurnberg, B.S.

Metabolic studies were made on the effects of salt restriction and the administration of mercurials on the electrolyte metabolism of patients in congestive failure. Varying degrees of hyponatremia and hypochloremic alkalosis were noted. Significant disturbances in potassium balance were not observed and normal potassium values were found in biopsy specimens of skeletal muscle. The alkalosis can be attributed to the effect of mercurials on chloride excretion which is relatively greater than their effect on sodium. The continued administration of mercurials resulted in electrolyte disturbances which prevented diuresis. When these abnormalities were corrected the mercurials again became effective.

IN RECENT years many reports have called attention to the occurrence of sodium chloride depletion* in patients with congestive heart failure.[1-4] This is of particular interest since previous studies had indicated that the untreated edematous cardiac patient retained sodium and water in amounts which maintained the serum sodium concentration within normal limits.[5, 6] A normal serum sodium has likewise been observed in preliminary observations on approximately 40 *untreated* patients.[7] Although these findings do not exclude the possibility that salt depletion can occur in untreated patients, they strongly suggest that depletion is most commonly associated with the various therapeutic regimens designed to treat edema.

Additional support for such a relationship is to be found in the increasing number of clinical reports on the appearance of dehydration and salt depletion following the use of mercurial diuretics.[8-12] The importance of assessing the possible role of therapy in the production of electrolyte abnormalities is further emphasized by the fact that mercurial diuretics, in large and frequent doses, have recently been advocated for the treatment of persistent and refractory decompensation.[13]

Since detailed metabolic data are not available, the present study was undertaken in an effort to define the nature and magnitude of electrolyte disturbances in a limited number of patients maintained on the conventional regimen of salt restriction combined with the frequent administration of mercurial diuretics. Electrolyte metabolism was studied in terms of (1) the over-all balance of sodium (Na), chloride (Cl), potassium (K), and nitrogen (N); (2) their respective serum concentrations; and (3) the electrolyte composition of skeletal muscle obtained by biopsy at the end of the balance study. Additional observations were made on the effectiveness of various procedures in correcting the electrolyte abnormalities.

Methods and Materials

Patient Selection. Each patient was selected from the medical wards of the Presbyterian Hospital; the criteria for selection included the presence of severe congestive heart failure with extensive peripheral edema, normal serum albumin, the absence of overt renal insufficiency, and the willingness to cooperate in a balance study which would include muscle biopsy. Seven patients were studied, aged 48 to 70 years. The etiology of the heart disease was: hypertension (2 cases), arteriosclerosis (3 cases), hypertension and arteriosclerosis (1 case), and rheumatic fever (1 case).

Procedure. All patients were transferred to the metabolism ward where they were treated through-

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York, N. Y.

This study was supported in part by a grant from the Cardiology Research Fund, Presbyterian Hospital.

During the course of the work, one of us (R. J. S.) was a National Heart Institute Trainee.

* In this paper the term electrolyte depletion is used to mean a decreased concentration in the body fluids; it does not refer to the total quantity present.

out the period of study with bed-rest and sedatives. Each patient was fully digitalized before the balance studies were begun and subsequently received constant maintenance doses. Mercuhydrin (the sodium salt of methoxyoximercuripropylsuccinylurea, Lakeside Laboratories, Milwaukee, Wis.) was administered intramuscularly. The dose of each injection was 2 cc. Daily weights were recorded routinely on the same scale in the fasting state. The patients were maintained on a constant low salt diet, the nature and quantity of which were selected by the patient during a preliminary period. The daily fluid intake of distilled water was likewise constant.

Two types of balance studies were carried out: complete (diet, urine and stools analyzed), and incomplete (urine analyzed, diet analyzed or calculated). In the four *complete* studies (patients J. C., G. M., W. M. and J. A.) divisions were made into arbitrary periods of seven to eight days during which the daily diet was kept constant by weight from identical food lots. Duplicate daily diets for each period were analyzed for sodium, chloride, potassium and nitrogen. At the end of each period the food lot was changed and duplicate diets were again analyzed for the subsequent period.* On three occasions, dietary intake was corrected for uneaten residues, the composition of which was determined by analysis. Consecutive 24 hour urines were collected, measured for volume, and analyzed daily for sodium, chloride and potassium. Urinary nitrogen values were obtained for each balance period from pooled aliquots of the daily urinary output. All stools were pooled for each period, and analyzed for sodium, chloride, potassium and nitrogen.

In the three *incomplete* balance studies (patients V. P., E. S. and S. M.) the identical procedure was carried out except that a few food lots were changed without analyzing each individual lot, and the stool electrolyte and nitrogen values were not determined. The dietary intake of patient S. M. was calculated from standard tables.[14-16]

Some patients received infusions of hypertonic sodium chloride, tablets of potassium chloride or ammonium chloride, and transfusions of whole blood. In each instance aliquots were analyzed, although nitrogen determinations were not made on the transfused blood.

Venous blood was drawn in the fasting state without the application of tourniquet when possible. Blood specimens were obtained about every two to three days; the blood values reported in table 1 were taken to coincide with the beginning and termination of each balance period.

Analytic Methods. Sodium and potassium were determined by internal lithium standard flame photometry.[17] The Volhard titration[18] was used for chloride analysis. Nitrogen determinations were done by the standard Kjeldahl procedure. Analyses on tissue, diets, and stool were done in triplicate. Serum carbon dioxide content was measured with the Van Slyke manometric apparatus. Serum urea nitrogen was determined by direct nesslerization according to the method of Gentzkow.[19] Arterial blood was collected in an airtight syringe to which had been added a small amount of heparin with sodium fluoride; pH was determined by a Cambridge glass electrode.

Diet and stool specimens were homogenized and made up to a known volume with distilled water. Aliquots for sodium and potassium determinations were digested until clear with equal volumes of concentrated nitric acid on an electric hot plate, filtered and made to volume with lithium for analysis by flame photometer. Chloride was determined on aliquots of the homogenate which had been digested on a boiling water bath for four to six hours with standard silver nitrate and concentrated nitric acid. Aliquots of the homogenate were also taken for nitrogen assay.

Muscle biopsies were obtained in the 4 patients in whom complete balance studies were done. The tissues were analyzed according to methods previously outlined,[20] with the one modification that the neutral fat extraction with ethyl ether was followed by an additional extraction with 100 per cent ethanol.

Calculations. In complete balance studies total balance figures for sodium, chloride, potassium and nitrogen represent the difference between intake and output. Intake was calculated as the sum of diet and medications. Output represents the sum of losses via urine, stool, and blood letting. Electrolyte content of whole blood was determined directly; nitrogen values were calculated.†

Previous studies have shown that a negative nitrogen balance is associated with a loss of potassium in amounts approximately proportional to the ratio of potassium to nitrogen in normal tissue. The potassium balance has, therefore, been corrected for the nitrogen balance, assuming a value of 2.5 mEq. potassium per Gm. nitrogen. Since the fluid

* Analyses of 21 diets are compared with values calculated from standard food tables[14-16]:

	Analyzed/Calculated Value	
	Mean	*Range*
Na	1.12	0.71–1.44
K	1.01	0.66–1.11
N	1.03	0.87–1.18

The ratios indicate a considerable variation in different food lots; the greater range observed for sodium is partly attributable to the low total content.

† Nitrogen content of whole blood was calculated on the basis of a constant ratio of 1 Gm. nitrogen per 2.5 mEq. of potassium.

balance can not be accurately obtained from measured intake and output due to the insensible loss, external fluid balance has been calculated from the observed changes in body weight which have been corrected for the loss or gain of tissue mass as determined by nitrogen balance. The factor of 30 Gm. nitrogen per Kg. wet tissue was used.

Results

Clinical Response

Of the 7 patients studied, 6 lost their edema and regained cardiac compensation. One patient (W. M., fig. 3) developed marked electrolyte abnormalities, failed to compensate, and subsequently died in congestive failure. Although all 7 patients developed abnormalities of electrolyte metabolism, in 3 instances (J. C., G. M. and J. A.) this did not preclude full clinical recovery. However, in 3 patients (V. P., M. S. and E. S.) cardiac decompensation persisted until the electrolyte abnormalities were corrected.

Patient W. M. complained of lassitude, drowsiness, weakness, and anorexia, but not of muscle cramps, during the third and fourth weeks of the balance study. These symptoms were alleviated following the administration of hypertonic saline on days 29 and 32. In the other patients of this series, as well as in many observed on the general medical wards, the absence of specific symptoms of sodium depletion has been striking. This experience is somewhat contrary to reports in the literature. Probably the degree and the duration of depletion are important in determining the resultant symptoms. It should be emphasized, however, that in heart failure, in contrast to adrenal cortical insufficiency, sodium depletion may be completely asymptomatic.

Electrolyte Balance

The results of the balance studies are recorded in tables 1, 2 and 3.* Graphs for each patient are presented in figures 1 to 6. The data indicate several different types of response to essentially the same regimen of salt restriction and mercurial administration. For purposes of exposition these responses have been arbitrarily divided into four phases.

1. *Minimal Electrolyte Abnormalities.* If the edematous patient were to excrete extracellular fluid and a proportional quantity of electrolytes, the serum electrolyte concentrations after diuresis would be maintained within normal limits. Such a response was observed in the early phases of treatment (approximately the first week) in patients J. C., G. M. and J. A. In these instances the high urine volume and rapid change in weight were associated with normal serum sodium and potassium concentrations and only slight changes in serum chloride and bicarbonate.

2. *Hypochloremic Alkalosis.* The second pattern observed was characterized by a fall in serum chloride and a reciprocal rise in serum carbon dioxide without significant change in sodium or potassium concentration. In some patients an elevation of arterial pH was also demonstrated. The development of alkalosis is best documented by the findings in J. A. (tables 1 and 2, fig. 4). The serum electrolytes were initially normal. At the end of 21 days of sodium chloride restriction and the repeated administration of mercurials the serum bicarbonate had risen to 30.7 mEq. per liter and the arterial pH was 7.50. During this period the serum potassium remained unchanged; the final serum sodium concentration was normal.

V. P. (fig. 5) showed a similar response. Prior to the detailed studies he had been treated on the general medical ward for five weeks with bed rest, digitalis, salt restriction, and mercurial diuretics, but was completely refractory to treatment. The balance data show that the administration of Mercuhydrin produced a metabolic alkalosis without achieving significant diuresis. The patient remained refractory to therapy until the alkalosis was treated with ammonium chloride. He then lost 11 Kg. of weight rapidly and regained complete cardiac compensation uneventfully. It is of interest that the diuresis was associated with a significant rise in serum sodium despite a constant low sodium intake; this was apparently the result of the relatively greater excretion of water than of sodium.

* Variations in stool output were quantitatively small and are not discussed in the text. However, the output of potassium and sodium in the stool represents a significant fraction of the total.

The pathogenesis of the alkalosis may be considered in reference to table 4 which summarizes the data for the period when mercurials were given approximately every other day and the low sodium chloride intake was maintained.

TABLE 1.—*Serum Electrolytes during Entire Period of Study*

Patient	Day	Wt.	Na	Cl	K	HCO_3	BUN	Hematocrit	Arterial pH
		Kg.	mEq./L.				mg.%	%	
J. C.	1	61.0	139.4	98.0	6.8	28.4	22	47.0	—
	8	56.0	133.8	96.1	5.1	—	—	45.9	—
	15	54.4	132.7	93.4	6.1	31.1	12	43.2	—
	22	54.4	130.0	91.3	5.2	—	—	42.2	—
	29	54.2	132.8	93.4	6.1	29.6	14	41.3	—
G. M.	1	65.8	129.0	98.2	5.0	26.2	21	43.0	—
	9	58.4	130.8	94.1	5.7	32.7	24	48.2	—
	16	52.6	132.0	90.2	5.2	31.2	27	51.8	—
	24	51.4	125.0	88.4	5.6	32.6	33	48.7	—
	31	50.8	122.8	88.6	5.7	30.4	37	46.7	—
	38	51.8	134.0	91.6	4.8	31.8	24	37.4	—
W. M.	1	64.2	131.0	78.7	4.0	37.8	26	43.4	7.49
	8	61.6	129.8	74.4	3.3	40.4	17	41.9	—
	15	59.8	128.2	70.1	2.5	42.3	19	42.1	—
	22	58.6	120.8	65.0	2.9	40.0	25	40.5	7.56
	29	63.0	117.8	66.0	4.4	34.9	42	48.1	—
	36	61.0	137.8	86.6	3.1	31.4	37	46.5	—
	43	62.5	128.5	74.0	2.7	36.6	31	44.1	—
	49	—	125.2	76.7	6.0	—	—	—	—
J. A.	1	86.0	143.9	104.2	5.0	26.7	19	50.2	—
	8	75.7	138.5	99.7	5.0	34.4	17	53.2	—
	15	72.2	137.3	92.0	5.1	32.7	20	56.7	—
	22	71.9	139.0	93.2	5.1	30.7	20	57.2	7.50
V. P.	1	89.2	133.9	95.9	4.9	28.6	11	—	7.50
	8	86.9	136.5	92.4	4.0	33.4	16	—	7.51
	15	83.1	139.7	103.3	3.8	25.8	20	—	7.47
	22	76.7	141.0	104.9	4.0	24.7	24	—	7.41
	29	76.1	137.3	101.7	4.1	24.6	17	—	7.40
S. M.	1	58.2	123.2	90.1	5.4	25.9	22	—	7.50
	6	56.5	133.5	94.4	5.1	27.3	22	—	7.50
E. S.	1	72.8	135.6	107.4	5.1	22.7	27	—	7.38
	8	55.6	131.9	103.2	4.6	22.1	53	—	7.39
	15	53.4	130.0	91.2	5.1	24.3	42	—	7.37

Patients G. M., W. M., and J. A. developed a significant alkalosis and were studied for a total of 72 days; mercurials were given for a total of 37 days. For the days on which mercury was administered the average urinary excretion of sodium and chloride was 101 and 110 mEq. per day respectively. The ratio of sodium to chloride in the urine was 0.92, which may be compared with the normal ratio of sodium to chloride of approximately 1.2 in the extracellular fluid (corrected for the Donnan factor). The data clearly indicate that the mercurials produced a relatively greater excretion of chloride than of sodium. Quantitatively this difference is sufficient to account for the resultant hypochloremic alkalosis. Compensatory renal mechanisms were ineffective presumably

TABLE 2.—*Electrolyte Intake and Output*[a]

Patient	Period	Days	Mercuhydrin	Δ Wt.	Fluid Intake	Urine Output	Sodium					Chloride					
							Intake		Output			Intake			Output		
							Diet	I.V.	Urine	Stool	Blood Letting	Diet	I.V.	Tablets	Urine	Stool	Blood Letting
			cc.	Kg.	cc.		mEq.		mEq.			mEq.			mEq.		
J. C.	I	1–7	4	−0.71	1500	1349	5.8	—	110.0	1.5	2.7	6.2	—	—	102.0	0.5	2.5
	II	8–14	4	−0.22	1500	1167	5.9	—	29.2	1.8	1.6	6.6	—	—	23.7	0.9	1.5
	III	15–21	4	0.0	1500	1017	6.5	—	8.7	1.3	0.7	7.9	—	—	9.8	0.02	0.7
	IV	22–28	—	−0.03	1500	1023	6.8	21.1[b]	4.6	0.5	1.4	7.8	21.1[b]	—	3.3	0.07	1.2
G. M.	I	1–8	6	−0.93	1550	1604	5.4	—	93.1	0.8	1.5	11.9	—	—	103.0	0.02	1.3
	II	9–15	8	−0.83	1550	2048	6.2	—	104.	1.4	1.1	14.6	—	—	94.2	0.09	1.0
	III	16–23	8	−0.15	1550	1547	8.3	—	32.6	1.1	1.5	15.9	—	—	37.2	0.08	1.3
	IV	24–30	6	−0.09	1550	1469	7.0	—	16.2	1.2	1.1	17.4	—	—	23.4	0.1	1.0
	V	31–37	2	+0.14	1550	1225	6.1	61.4[c]	33.1	7.0	2.3	23.7	61.4[c]	—	57.7	0.3	2.0
W. M.	I	1–7	6	−0.37	1500	1298	6.3	—	32.4	1.3	1.5	13.8	—	—	45.3	0.7	1.2
	II	8–14	8	−0.26	1500	1141	8.1	—	26.3	1.7	1.4	12.8	—	—	35.7	0.2	1.1
	III	15–21	12	−0.03	1500	1205	6.6	—	29.0	0.5	1.9	14.3	—	—	32.5	0.2	0.9
	IV	22–28	0	+0.63	1500	743	5.3	38.1[d]	2.4	1.1	1.5	10.2	32.7[d]	18.8[e]	3.1	0.3	1.2
	V	29–35	4	−0.29	1500	1367	6.7	186.0[g]	47.1	0.8	2.2	10.1	186.0[g]	—	68.1	0.1	1.9
	VI	36–42	6	+0.21	2286[h]	2029	8.4	99.0[i]	114.0	1.0	1.5	16.1	99.0[i]	—	141.2	0.04	1.2
	VII	43–48	2	+0.30	2600	1083	7.6	—	9.3	2.0	1.8	9.2	—	60.3[j]	18.2	0.2	1.4
J. A.	I	1–7	6	−1.47	1520	1863	14.1	—	172.0	0.8	1.2	24.4	—	—	156.0	0.2	1.2
	II	8–14	8	−0.50	1520	1255	14.1	—	61.7	1.4	1.0	22.9	—	—	77.7	0.5	1.0
	III	15–21	6	−0.04	1520	1063	12.4	—	11.3	0.6	1.5	22.2	—	—	20.1	0.6	1.5
V. P.	I	1–7	8	−0.33	1500	1467	12.9	—	23.0	—	—	19.1	—	—	54.4	—	—
	II	8–14	8	−0.54	1500	2259	12.9	—	92.6	—	—	19.1	—	108.	168.	—	—
	III	15–21	6	−0.91	1500	1753	12.9	—	83.5	—	—	19.1	—	108.	167.	—	—
	IV	22–28	8	−0.09	1500	1504	12.9	—	44.8	—	—	19.1	—	108.	140.	—	—
S. M.	I	1–6	6	−0.28	1500	1569	8.0	117.0[k]	86.4	—	—	14.6	117.[k]	—	134.	—	—
E. S.	I	1–7	6	−2.46	1500	3083	2.0	—	330.	—	—	19.5	—	96.1[l]	387.	—	—
	II	8–14	2	−0.31	1500	782	6.8	—	9.5	—	—	16.5	—	—	18.0	—	—

Patient	Period	Days	Potassium						Nitrogen				
			Intake			Output			Intake		Output		
			Diet	I.V.	Tablets	Urine	Stool	Blood Letting	Diet	Tablets	Urine	Stool	Blood Letting
			mEq.			mEq.			Gm.		Gm.		
J. C.	I	1–7	39.4	—	—	38.3	4.2	1.6	4.7	—	6.0	0.5	0.6
	II	8–14	50.0	—	—	44.1	3.3	0.9	4.9	—	6.2	0.8	0.4
	III	15–21	46.4	—	—	43.3	4.4	0.4	5.3	—	5.0	0.5	0.2
	IV	22–28	45.2	—	—	43.4	4.2	0.7	5.3	—	4.9	0.5	0.3
G. M.	I	1–8	44.0	—	—	45.6	4.4	0.9	8.1	—	8.5	0.5	0.4
	II	9–15	50.5	—	—	54.0	9.9	0.7	8.2	—	8.9	1.1	0.3
	III	16–23	51.4	—	—	45.4	4.6	0.9	9.0	—	9.4	0.5	0.4
	IV	24–30	53.6	—	—	49.0	7.0	0.7	8.5	—	9.2	0.9	0.3
	V	31–37	52.4	—	—	66.7	10.1	1.1	10.9	—	8.9	1.4	0.5
W. M.	I	1–7	42.2	—	—	51.9	5.0	0.9	5.6	—	7.5	1.0	0.4
	II	8–14	45.2	—	—	45.3	8.4	0.9	7.2	—	7.1	1.0	0.3
	III	15–21	44.0	—	—	46.9	3.4	0.8	6.9	—	7.7	0.6	0.3
	IV	22–28	42.5	17.1[d]	18.8[e]	51.4	12.3	0.9	—	6.8[f]	—	—	0.4
	V	29–35	46.4	—	—	58.5	9.8	1.1	6.7	—	7.7	1.0	0.4
	VI	36–42	55.9	—	—	52.5	8.9	0.9	7.1	—	11.4	1.1	0.3
	VII	43–48	60.1	—	60.3[i]	41.4	15.0	1.1	6.7	—	5.6	1.4	0.4
J. A.	I	1–7	74.8	—	—	86.6	6.3	1.0	10.5	—	18.5	0.9	0.4
	II	8–14	77.2	—	—	79.9	7.8	0.9	11.5	—	14.2	1.6	0.3
	III	15–21	80.0	—	—	76.8	5.1	1.1	11.2	—	12.6	0.7	0.4
V. P.	I	1–7	76.5	—	—	70.2	—	—	12.3	—	7.7	—	—
	II	8–14	76.5	—	—	82.9	—	—	12.3	1.5	12.4	—	—
	III	15–21	76.5	—	—	71.0	—	—	12.3	1.5	14.1	—	—
	IV	22–28	76.5	—	—	67.4	—	—	12.3	1.5	13.5	—	—
S. M.	I	1–6	61.6	—	—	58.8	—	—	—	—	—	—	—
E. S.	I	1–7	47.3	—	—	59.8	—	—	9.1	1.3[l]	10.2	—	—
	II	8–14	34.6	—	—	32.2	—	—	7.3	—	9.9	—	—

[a] All results calculated as average 24 hour values for period except Mercuhydrin dosage which is given as total for period. [b] 148 mEq. intravenously as 5 per cent sodium chloride, day 26. [c] 430 mEq. intravenously as 5 per cent sodium chloride, day 33. [d] Transfusion on days 25, 26 and 27, 1000 cc. whole blood each day. [e] 65.1 and 66.6 mEq. by mouth on days 23 and 24. [f] By transfusion. [g] 431, 437 and 437 mEq. intravenously as 5 per cent sodium chloride, days 29, 32 and 35. [h] Fluid intake increased from 1500 cc. to 2600 cc. on day 38. [i] 262 and 431 mEq. intravenously as 5 per cent sodium chloride, days 38 and 41. [j] 50, 48, 132 and 132 mEq. by mouth, days 45, 46, 47 and 48. [k] 700 mEq. intravenously as 5 per cent sodium chloride, day 4. [l] 6 Gm. NH_4Cl days 1 to 6, 1.5 Gm., day 7.

because of either the low sodium chloride intake or the frequent administration of the mercurial.

sure was within normal limits. This would suggest that, due to the associated cardiac decompensation, some of the patients were

TABLE 3.—*Total Balances of Four Patients Treated with Salt Restriction and Mercurials*[a]

Patient	Periods	Number of Days	△ Weight	Corrected Fluid Balance[b]	Na	K	Cl	N	Corrected K Balance[b]	Na Balance	Cl Balance
										Corrected Fluid Bal.	Corrected Fluid Bal.
			Kg.	liters	mEq.			Gm.	mEq.	mEq./L	
J. C.	I–III	21	−6.6	−5.5	−972	−32	−848	−33.7	+52	177	154
G. M.	I–IV	30	−15.0	−13.4	−1719	−174	−1539	−48.1	−54	128	115
W. M.	I–III	21	−5.6	−4.1	−525	−225	−539	−44.5	−114	128	132
J. A.	I–III	21	−14.1	−10.2	−1472	−237	−1325	−115.8	+53	144	130

[a] Includes only the balance data for periods prior to attempts to correct electrolyte deficits, that is, while on low sodium chloride intake.

[b] For calculation see text.

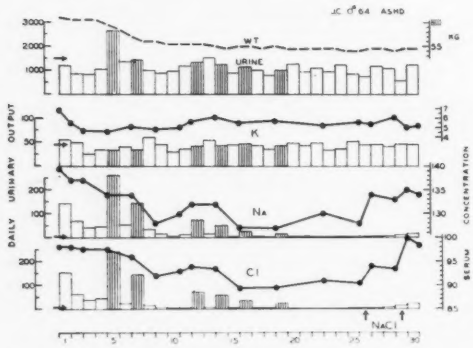


FIG. 1. Patient J. C. Unit #975984. Arteriosclerotic heart disease; congestive failure of two months duration, treated with digitalis and potassium citrate prior to admission, dosages unknown. Asymptomatic and clinically free of edema by day 8. Muscle biopsy on day 23. 175 and 150 cc. 5 per cent sodium chloride intravenously, days 26 and 29, respectively.

In all figures: Daily urinary excretion indicated by blocks, electrolytes as mEq. and urine volume in cc. shown on scale to left. Serum electrolytes (solid lines) on scale to right, mEq. per liter. Constant dietary and fluid intake indicated by horizontal arrows on left. Vertical stripes indicate days on which mercurials were administered.

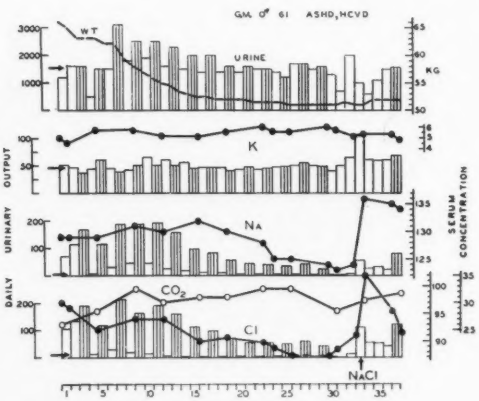


FIG. 2. Patient G. M. Unit #717239. Arteriosclerotic and hypertensive heart disease; recurrent congestive failure of two years duration, frequent hospitalizations. Asymptomatic and clinically free of edema by day 13. Muscle biopsy on day 31. On day 33, 500 cc. 5 per cent sodium chloride intravenously. See figure 1 for explanation.

In 4 of the patients with hypocholoremia the arterial pH was elevated. In one of these patients the carbon dioxide partial pressure was elevated while in 3 others the partial pressure was within normal limits. This would suggest that, due to the associated cardiac decompensation, some of the patients were unable to compensate for their metabolic alkalosis by hypoventilation.

3. *Depletion of Both Chloride and Sodium.* The diuresis of cardiac edema may also be associated with the urinary excretion of both sodium and chloride in excess of the water lost, leading to a decreased serum concentration of both electrolytes. A parallel depletion of sodium and chloride was observed in J. C., while in G. M. and W. M. the chloride depletion was greater and resulted in alkalosis. It

TABLE 4.—*Average Daily Urine Electrolyte Excretion. Output on Days Patients Received Mercurial Compared with Nonmercurial Days. All Patients on Constant Diet*

Patient	Period	Number Days		Urine Volume		Na		Cl		K	
		Mercurial	Non-mercurial	Mercurial	Non-mercurial	Mercurial	Non-mercurial	Mercurial	Non-mercurial	Mercurial	Non-mercurial
				cc./24 hrs.		mEq./24 hrs.					
J. C.	I–III	6	15	1480	1060	95	30	97	28	40	43
G. M.	I–IV	14	16	1900	1450	103	26	113	23	48	49
W. M.	I–III	13	8	1410	900	42	8	54	12	53	40
J. A.	I–III	10	11	1860	970	157	19	162	15	92	72
V. P.	I–IV	15[a]	13[a]	2210	1190	106	10	208	45	102	39
E. S.	I	3[b]	4[b]	4450	2060	482	216	550	265	60	60
S. M.	I	3	3	1920	1210	142	31	214	53	57	61

[a] Includes 11 nonmercurial and 9 mercurial days while receiving NH_4Cl.
[b] Receiving daily NH_4Cl.

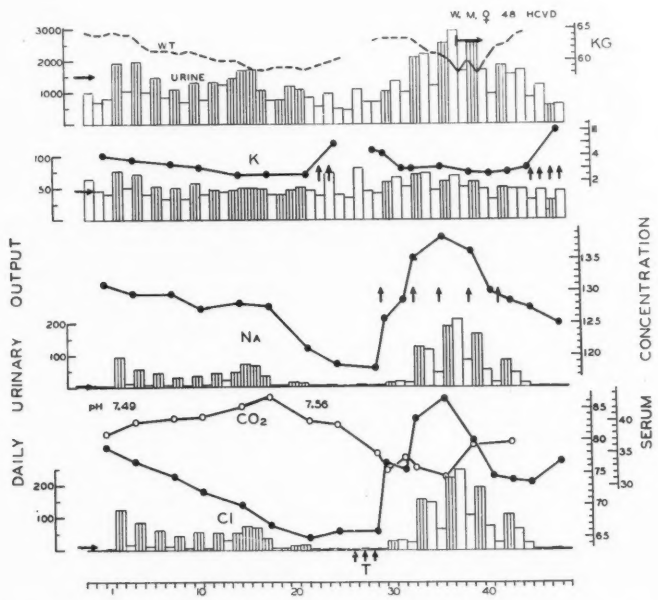


FIG. 3. Patient W. M. Unit #893534. Hypertension for nine years; persistent congestive failure for two years, refractory to bed rest, salt restriction, digitalis and repeated mercurial injections. Estimated 25 Kg. of edema fluid at start of study. Never regained cardiac compensation. Muscle biopsy on day 22. Oral potassium chloride on days 23, 24, 45, 46, 47 and 48 indicated by vertical arrows in K columns. Transfusions of whole blood, 1 liter each, on days 25, 26 and 27 indicated by vertical arrows marked T. Five per cent sodium chloride intravenously on days 29, 32, 35, 38 and 41, represented by vertical arrows in Na columns, amounting to 500, 500, 500, 300 and 500 cc. respectively. Daily fluid intake increased from 1500 cc. to 2600 cc. on day 38. See figure 1 for explanation. pH values refer to arterial blood.

should be pointed out that in J. C. and G. M. the serum concentration did not fall appreciably until after cardiac compensation had been restored. On the other hand, in W. M. and S. M. the depression of serum sodium and chloride occurred while the patients were still severely decompensated.

4. *Potassium Balance.* Mercurial diuretics

have been shown to increase the renal excretion of potassium in the dog[21-23] as well as in man.[24] In the present study this effect was found to be variable when measured in terms of the 24 hour output (table 4). In 4 patients there was no change in potassium excretion irrespective of changes in urine volume. In W. M. and J. A. Mercuhydrin slightly increased potassium excretion, while in V. P. the effect was marked. No explanation is apparent for the differences in response. A decreased serum potassium concentration was observed in only one patient (W. M.)

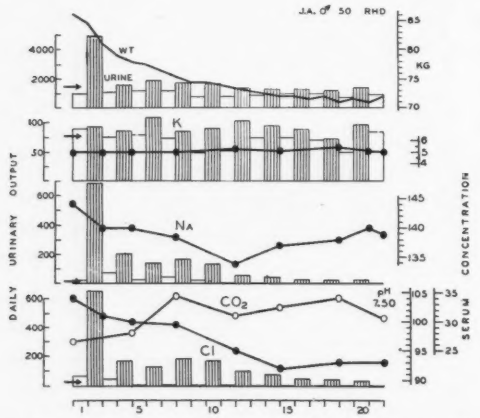


FIG. 4. Patient J. A. Unit #970044. Rheumatic heart disease; intermittent congestive heart failure for one year, always responding to bed rest, digitalis, salt restriction, and mercurials. No evidence of rheumatic activity. Clinically free of edema on day 10. Muscle biopsy on day 21. See figure 1 for explanation.

In J. A. mercurials increased potassium excretion but a normal balance was maintained by potassium conservation on the days when mercurials were not given (tables 3 and 4). Throughout the study his serum potassium remained normal and there were no abnormalities in tissue electrolytes as determined by muscle biopsy. In contrast, W. M. was apparently unable to conserve potassium. The serum concentration fell from 4.0 to 2.5 mEq. per liter over a three week period during which time the corrected potassium balance was −114 mEq. with a total dietary intake of 920 mEq. A muscle biopsy obtained on day 22 revealed only borderline changes in potassium content.*

Intracellular Cation Concentrations

Changes in the sodium and potassium concentrations of the intracellular space can be evaluated by a variety of methods. It was found that calculation of the balance data by the method of Darrow[25] was not completely satisfactory for the present studies for two reasons: first, the apparent changes were relatively small and had developed over a long period of time, and second, the calculations require a knowledge of the initial extracellular fluid volume which, in the edematous patient, can neither be measured with great accuracy[26] nor assumed with any validity. Direct analyses of red blood corpuscles were periodically performed in several patients by the determination of whole blood and plasma electrolytes and the calculation of the red cell composition on the basis of the hematocrit. The results were inconsistent, showed no specific trends, and are not reported.

Since skeletal muscle represents the major portion of the intracellular space it was hoped that direct analyses of biopsy specimens might yield helpful information. The results obtained in 4 patients are given in table 5*A*; the corresponding values of serum electrolytes are given in table 5*B*. The analytic data are presented in terms of the observed whole tissue values; the intracellular concentrations have also been calculated on the basis of the chloride space. Recent studies[27] show that the chloride space is not an accurate index of the total extracellular space of the body. However, since available evidence indicates that only a small fraction of skeletal muscle chloride is intra-

* Presumptive evidence of potassium depletion was obtained during days 23 to 28 when the patient was given 252 mEq. potassium (orally and by transfusion) of which approximately 100 mEq. were retained. The patient's course was complicated by bleeding into the soft tissues of the leg and a transfusion reaction. In addition, a complete nitrogen balance was not obtained for this period. The balance data can therefore be considered as only approximate values.

TABLE 5.—*A. Electrolyte Composition of Skeletal Muscle*

Patient	Specimen	Muscle Composition							Calculated Values					
		Fat	H_2O	N	Cl	Na	K	K	ECW	ICW	Na	K	Na + K	N
		%	Gm.*	Gm.	mEq.				ml.	ml.	mEq.			Gm.
		Fresh Tissue	per Kg. fat free tissue					per 100 Gm. fat free solids	per Kg. fat free tissue		per L. intracellular water			
Controls[a]	Skel. M.	3.6	793	31.7	28.4	39.7	85.7	41.3	253	540	6	157	163	58.7
J. C.	Gastroc.	5.2	795	29.2	21.1	35.4	87.0	42.5	204	591	13	145	158	49.4
G. M.	Gastroc.	2.7	784	30.3	20.4	27.2	72.8	33.7	202	582	3	123	126	52.2
W. M.	Quadriceps	2.6	844	22.7	30.0	64.5	64.4	40.8	408	436	32	145	177	52.2
J. A.	Gastroc.	4.1	765[b]	33.3	20.2	35.9	104.4	44.3	184	581	15	177	192	76.3

B. Serum Values Obtained at Time of Muscle Biopsy

Patient	Balance Day	Specimen	Anesthesia	Serum Concentration							Arterial pH
				H_2O	Protein	HCO_3	Cl	Na	K	Urea N	
				%	%	mEq./L.				mg. %	
Controls[a]	—	—	—	91.2	7.2	24.8	96.7	135	4.2	—	—
J. C.	23	Gastroc.	Procaine, local	91.6	7.1	29.6	90.3	130	5.8	14	—
G. M.	31	Gastroc.	Procaine, local	91.2	6.8	28.5	87.5	121	6.0	37	—
W. M.	22	Quadriceps	Procaine, local	91.6	6.6	40.0	64.2	120	2.6	25	7.56
J. A.	22	Gastroc.	Sciatic block	90.4	—	33.9[c]	94.2	140	5.1	24	7.50

For calculations, see text.

[a] Control data from reference 20.

[b] Apparent low water content probably due to fluid evaporation during preparation of tissue.

[c] Serum bicarbonate value two days before biopsy.

cellular,[28] and since no studies have shown that this fraction is subject to variation, the intracellular calculations are presented, based on the assumed identity of the chloride and extracellular spaces. The calculations must obviously be considered as tentative, not only because of the basic assumptions involved, but also because of the fact that relatively small analytic errors are associated with large changes in the calculated values.

Considered in view of the above limitations, the data of table 5 show that the muscle water and extracellular space of patients J. C., G. M. and J. A. were not increased, a finding consistent with the absence of demonstrable edema at the time the specimens were obtained. W. M. was markedly edematous and this is reflected in an increase in the calculated extracellular water of the muscle.

The absolute potassium content of muscle

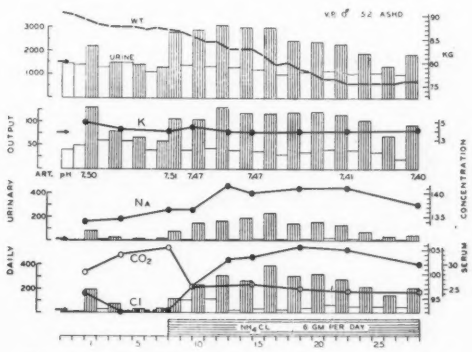


FIG. 5. Patient V. P. Unit #006553. Arteriosclerotic heart disease; intermittent congestive failure for two years. Prior to balance study treated on general medical ward for five weeks with bed rest, digitalis, salt restriction and mercurials without restoration of cardiac compensation. Asymptomatic and clinically free of edema by day 22 of balance study. See figure 1 for explanation. pH values refer to arterial blood.

tissue is probably most accurately described with reference to fat-free solids. As seen in table 5, no examples of depletion are noted except for G. M., whose tissue potassium is 18 per cent below the controls. Comparison of

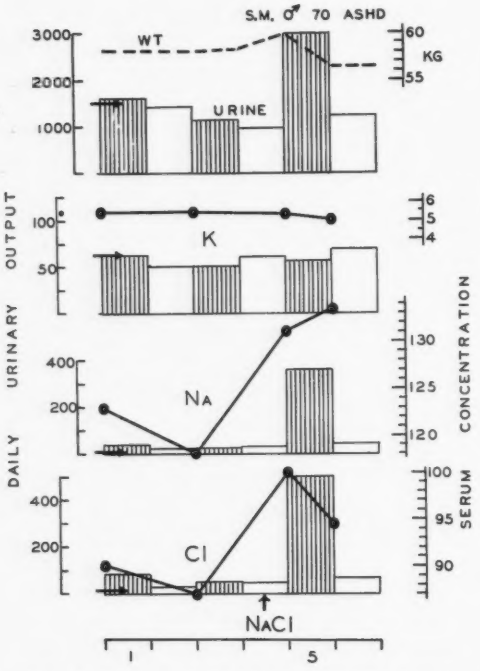


FIG. 6. Patient S. M. Unit #557458. Arteriosclerotic heart disease; congestive failure of two months duration. Prior to study treated on general medical ward for four weeks with bed rest, digitalis, salt restriction and mercurials; hyponatremia and hypochloremia resulted without loss of edema. Two weeks before balance study received 500 cc. 5 per cent sodium chloride intravenously followed by marked diuresis in response to mercurial. Mercuhydrin administration continued with recurrence of edema, sodium chloride depletion, and loss of response to mercury. Balance study begun when moderate amount of edema persisted. On day 4, 820 cc. 5 per cent sodium chloride intravenously. Clinically free of edema on day 6. See figure 1 for explanation.

these results with the balance data show a satisfactory agreement, although an exact comparison is limited in part by the fact that the muscle composition at the start of the balance study is not known. J. C. and J. A. were in slightly positive potassium balance (table 3). G. M. had a corrected potassium balance of −54 mEq. over a 30 day period (total intake 1492 mEq.). W. M. had a corrected potassium balance of −114 mEq. for 21 days (total intake 920 mEq.), and in addition developed a low serum potassium concentration.

The calculated intracellular values reveal no increase in intracellular sodium except in the case of W. M., in whom the intracellular sodium and sum of sodium and potassium are abnormally high. This was previously noted in metabolic alkalosis[20] but its significance is obscure.

In general, the muscle analyses failed to show significant changes in intracellular cation concentrations. It may be emphasized that these data do not indicate whether or not specific changes may have occurred in other tissues.

Nitrogen Metabolism

All 4 patients on whom complete balance data were obtained were in significantly negative nitrogen balance (table 3). Dietary nitrogen was low and was thus comparable to the intake of the severely decompensated patient in whom anorexia is an important symptom.

Serum Urea Nitrogen

The effect of the therapeutic regimen on the level of serum urea nitrogen was variable. Of the 7 patients, only one developed appreciable nitrogen retention.* The serum urea nitrogen of E. S. rose from an admission value of 27 mg. per 100 cc. to 53 mg. per 100 cc. at the end of eight days. This change was accompanied by the development of nausea, marked anorexia, and generalized lassitude. During this period the patient had received four injections of Mercuhydrin and for the first six days of the study had been given 36 Gm. of ammonium chloride. The elevation of urea nitrogen occurred in association with an 18 Kg. diuresis and the complete restoration of cardiac compensation without the development of significant abnormalities of the serum electrolytes. When mercurials and ammonium chloride were

* W. M. had a single transient rise in serum urea nitrogen to 52 mg. per 100 cc. following a transfusion reaction. This rapidly subsided.

discontinued, the patient was maintained on a low salt diet and digitalis without other medication. Elevation of the serum urea nitrogen persisted and was not alleviated by forcing fluids up to 2600 cc. daily. After two weeks the serum sodium and chloride concentrations fell to 125 and 85 mEq. per liter, respectively. Because the balance study had been previously discontinued, the explanation for this salt depletion is not apparent. However, in response to the infusion of 600 cc. of 5 per cent sodium chloride, the patient's electrolyte deficits were immediately corrected. Five days later his serum urea nitrogen had fallen from 40 mg. per 100 cc. to 22 mg. per 100 cc. and he was completely asymptomatic.

Correction of Electrolyte Abnormalities

The administration of certain salts for the correction of electrolyte deficits was studied with particular emphasis on their effect upon the responsiveness of the patient to mercurial diuretics. Three agents were employed—hypertonic sodium chloride infusions, ammonium chloride, and potassium chloride.

Hypertonic sodium chloride (5 per cent) was given intravenously ten times to 5 patients in amounts from 150 to 820 cc. per infusion. In six instances the procedure was carried out cautiously while the patient was still in congestive heart failure. No increase in peripheral venous pressure or signs of left ventricular failure was noted. Minor unpleasant effects were sometimes encountered, such as headache, a sensation of burning along the course of the vein, and local venous thrombosis (one case).

The infusion of 5 per cent sodium chloride per se had a small and variable effect upon urinary excretion during the subsequent 24 hour period. Urine volume usually decreased; sodium and chloride excretion increased slightly in 3 patients; potassium excretion increased in one patient.

A single infusion of 500 cc. of 5 per cent sodium chloride elevated the serum sodium and chloride 7 to 10 mEq. per liter; variations in response depended partly upon the amount of edema present. A sharp fall in hematocrit of approximately 25 per cent was consistent with an expansion of the plasma volume. A major factor conditioning the response of the patient appeared to be the appearance of marked thirst. W. M. (fig. 3) received three infusions of hypertonic sodium chloride from days 29 to 35. There was an associated rise in serum sodium from 117 to 138 mEq. per liter, and the patient, formerly refractory to mercurials, again responded to their administration. Severe thirst was experienced when the serum sodium had risen to 125 mEq. per liter and this became so distressing that the fluid intake was increased on day 38. A total of 800 cc. of 5 per cent sodium chloride was given intravenously during the following four days. But the total intake for this period, including food and fluids by mouth, was 726 mEq. of sodium and 10,400 cc. water—a ratio of 70 mEq. per liter. The effective sodium chloride intake was, therefore, *not* hypertonic. This was associated with a rapid accumulation of edema, gain in weight, reduction in serum sodium and chloride concentrations, and the loss of responsiveness to Mercuhydrin.

The effect of the administration of hypertonic saline and the simultaneous restriction of fluids on the response to Mercuhydrin is best illustrated by the data on S. M. (fig. 6). The patient was refractory to Mercuhydrin when the serum sodium and chloride were about 120 and 88 mEq. per liter, respectively. The infusion of 820 cc. of 5 per cent sodium chloride on day 4 was not associated with any immediate change in renal excretion, but the injection of Mercuhydrin on the subsequent day, when the serum sodium and chloride were 130 and 100 mEq. per liter, respectively, was followed by a diuresis of sodium, chloride, and water and a 3 Kg. loss of weight. This response was observed twice in this patient, although documented by balance study only once. A similar response was transiently observed in W. M. (described above).

Hypertonic sodium chloride was given to 4 other patients (not included in this series) in whom the failure to respond to mercurials had been associated with sodium depletion. Detailed observations were not made but in each instance the infusion was followed by thirst, an increase in water intake, the accumulation

of edema, and no significant change in the response to mercurials.

Ammonium chloride was given to one patient (V. P., fig. 5) with hypochloremic alkalosis and a slight depression of serum sodium concentration. This was followed by a rapid return of the serum electrolyte concentrations toward normal. While in alkalosis he had been refractory to mercury; a satisfactory diuresis was immediately obtained when the alkalosis was corrected.

Because of the reported association of potassium depletion and alkalosis,[29] patient W. M. was given potassium chloride on two occasions in the hope that it might correct the alkalosis. On both occasions the serum potassium rose to normal levels, but no clinical improvement was noted and the patient remained refractory to mercurials. The experience with this patient certainly does not constitute a fair trial for potassium therapy in mercurial induced alkalosis; the problem will be discussed more fully below.

Discussion

These observations were made to ascertain the electrolyte abnormalities that might accompany congestive heart failure when treated with digitalis, low sodium chloride intake, and the frequent administration of mercurial diuretics. The results do not necessarily apply to other methods of management or to patients receiving either more liberal allowances of dietary sodium chloride or smaller or less frequent injections of mercurials. However, the dosage of Mercuhydrin employed in this series was considerably less than the maximum schedule recommended for refractory patients by Gold, namely, 2 cc. every 12 hours.[13] In some patients the observed abnormalities were undoubtedly magnified by the fact that the regimen was continued for a considerable length of time after all signs of decompensation had cleared. However, it should be emphasized that the most severe examples of alkalosis and sodium depletion occurred in the presence of persistent decompensation with massive edema.

The data emphasize certain variabilities in response. The smaller deviations in the serum electrolyte pattern were noted when Mercuhydrin produced a prompt diuresis, while the continued administration of mercurials was associated with hypochloremic alkalosis, and in most patients with sodium depletion. Some of these variations in patient response can not be adequately explained. The possibility arises that electrolyte depletion in cardiacs might be associated with overt renal insufficiency. In the patients of this series the highest initial serum urea nitrogen was 27 mg. per 100 cc.; phenolsulfonphthalein excretion was normal; and the maximum specific gravity of the urine was 1.020 or higher. Albuminuria was variable and, in the presence of congestive failure, can not be considered indicative of specific renal disease. Intravenous pyelograms were normal. Thus, by conventional clinical criteria, the patients did not have renal failure. These findings serve to emphasize that the role of the kidney in regulating the electrolyte composition of the body fluids can not be accurately evaluated by the usual clinical tests of renal function.

A number of reports have previously shown that the administration of mercurials is associated with a greater increase in the urinary excretion of chloride than of sodium.[30–34] Varying degrees of hypochloremia have also been noted.[30, 31, 34-37] The possible relationship of mercurial-induced alkalosis to disturbances in potassium metabolism deserves particular attention. Darrow and his associates have observed that metabolic alkalosis may be associated with a depletion of intracellular potassium and a corresponding increase in intracellular sodium in skeletal muscle.[38] This has been confirmed for man in patients with gastric alkalosis.[20] The clinical importance of these observations lies in the fact that the administration of potassium salts is extremely effective in correcting the alkalosis, whereas sodium salts and ammonium chloride are relatively ineffective.[29] It is obviously important to determine whether mercurial-induced alkalosis is accompanied by similar changes and whether potassium should be used for its treatment. Our studies do not demonstrate a significant potassium deficit. The pertinent observations are: (1) the corrected potassium balance was

slightly positive in one case and slightly negative in 2 cases with alkalosis; (2) three of four muscle biopsies showed a normal content of potassium on a dry weight basis; (3) a slight increase in the calculated intracellular sodium content was observed in only one of four muscle biopsies; and (4) the discrepancies between the external balance of sodium and chloride can be readily accounted for by the resultant changes in extracellular concentrations and do not indicate an intracellular migration of sodium. In addition, in V. P. the alkalosis was easily corrected by ammonium chloride; the administration of potassium salts to W. M. had no effect on either the alkalosis or the clinical course. It is realized that a single therapeutic trial with potassium salts is inconclusive; they were not given to subsequent patients because the accumulated data failed to indicate a rationale for their administration. It is, of course, possible that in a larger series of patients instances of significant potassium depletion might be encountered.

The physiologic factors modifying the diuretic response to organic mercurials have been summarized in an excellent review by Pitts and Sartorius.[39] The refractory state has been associated with (1) metabolic alkalosis and/or a decreased serum chloride concentration[40-41]; (2) dehydration; and (3) a decreased rate of glomerular filtration.[42] A decrease in either the concentration or the absolute quantity of chloride in the glomerular filtrate appears to be a common factor, but detailed mechanisms are not known. Our data document a sequence of events associated with the continued administration of mercurials, namely, excessive loss of chloride in the urine, hypochloremic alkalosis (with or without hyponatremia), failure to respond to mercurials, correction of the alkalosis, and return of diuretic response to mercury.*

The combined administration of ammonium chloride and mercurials has been based upon their synergistic diuretic action.[40] It is not determined whether this effect is due to the resultant acidosis[40] or to the high serum chloride concentration per se.[43] The production of hypochloremic alkalosis by prolonged mercurial therapy emphasizes another rationale for the adjuvant use of ammonium chloride, namely, that it prevents the development of alkalosis, or corrects it if present. Excessive administration of ammonium chloride should obviously be avoided because of the potential danger of producing a severe acidosis.

It is difficult to assess the value of hypertonic sodium chloride for the correction of sodium chloride depletion. Although a satisfactory clinical response was clearly demonstrated in three instances, other considerations indicate that hypertonic sodium chloride is not the agent of choice. First, its administration is accompanied by the potentially hazardous expansion of plasma volume. Second, it produces considerable thirst leading to an increased water intake which may partially or completely nullify its effects. A good clinical response was obtained only when water was rigidly restricted. And third, in the edematous patient with hyponatremia the total quantity of sodium in the extracellular fluid is increased and is potentially available for the correction of a low concentration. As illustrated by patient V. P., the initiation of a diuresis can correct hyponatremia without an increase in sodium intake.

These studies should not be interpreted as questioning the value of the mercurial diuretics in the management of cardiac decompensation. They emphasize, however, that the continued administration of mercury may, in some patients at least, result in severe disturbances of electrolyte metabolism, and that these disturbances themselves prevent a satisfactory diuresis. In the edematous patient who has become refractory to a mercurial because of hypochloremic alkalosis, the administration of the drug apparently may be effectively continued provided the electrolyte abnormalities are corrected by appropriate therapy.

Summary

1. The effects of salt restriction and mercurial diuretics on electrolyte metabolism were

* This sequence of events has also been noted in studies reported in abstract by Schwartz, W. B., and Wallace, W. M.: J. Clin. Investigation **29**: 844, 1950.

studied in patients with congestive heart failure. Complete balances for sodium, chloride, potassium and nitrogen were obtained in 4 patients; in 4 patients, specimens of skeletal muscle obtained by biopsy were analyzed; less complete balance data were obtained in 3 other patients.

2. Electrolyte disturbances included hypochloremic alkalosis and varying degrees of sodium depletion. No evidence was found for the appearance of an intracellular potassium deficit, although one patient developed a low serum potassium and was in slightly negative potassium balance.

3. In 3 patients electrolyte abnormalities did not prevent recovery from congestive heart failure, while in 3 others they were directly correlated with the persistence of cardiac edema.

4. Limited observations on corrective therapy were made. The significance of hypochloremic alkalosis occurring in the course of treatment with mercurial diuretics is discussed.

Acknowledgments

We are indebted to Miss Ann D. Barrows, dietician, and to Miss Margaret C. Hawthorne, head nurse, for their invaluable assistance.

REFERENCES

[1] Peters, J. P.: The role of sodium in the production of edema. New England J. Med. **239:** 353, 1918.

[2] Schroeder, H. A.: Renal failure associated with low extracellular sodium chloride. J.A.M.A. **141:** 117, 1949.

[3] Merrill, A. J.: Mechanisms of salt and water retention in heart failure. Am. J. Med. **6:** 357, 1949.

[4] Fox, C. L., Jr., Friedburg, C. K., and White, A. G.: Electrolyte abnormalities in chronic congestive heart failure; effects of administration of potassium and sodium salts. J. Clin. Investigation **28:** 781, 1949.

[5] Atchley, D. W., Loeb, R. F., Benedict, E. M., and Palmer, W. W.: Physical and chemical studies of human blood serum. III. A study of miscellaneous disease conditions Arch. Int. Med. **31:** 616, 1923.

[6] Peters, J. P., and Van Slyke, D. D.: Quantitative clinical chemistry. Vol. I. Interpretations. Baltimore, Williams & Wilkins, 1931.

[7] Stock, R. J., and Mudge, G. H.: Unpublished data.

[8] Poll, D., and Stern, J. E.: Untoward effects of diuresis. Arch. Int. Med. **58:** 1087, 1936.

[9] Klinghoffer, K. A.: Dehydration from diuretics. New Internat. Clinics **1:** 221, 1941.

[10] MacGuire, W. B.: Risk of uremia due to sodium depletion. J.A.M.A. **137:** 1377, 1948.

[11] Soloff, L. A., and Zatuchni, J.: Syndrome of salt depletion. J.A.M.A. **139:** 1136, 1949.

[12] Jaffee, H. L., Master, A. M., and Dorrance, W.: The salt depletion syndrome following mercurial diuresis in elderly persons. Am. J. M. Sc. **220:** 60, 1950.

[13] Gold, H., Kwit, N. T., Modell, W., Hanlon, L. W., Kramer, M., Greenberg, S., Otto, H. L., Cotlove, E. W., Benton, J. C., Pearlmutter, M., and Zahm, W.: A system for the routine treatment of the failing heart. Am. J. Med. **3:** 665, 1947.

[14] Sherman, H.: Chemistry of Food and Nutrition, ed. 7. New York, Macmillan, 1945.

[15] Bills, C. E., McDonald, F. G., Niedermeier, W., and Schwartz, M. C.: Sodium and potassium in foods and water. J. Am. Dietetic A. **25:** 304, 1949.

[16] Chatfield, C., and Adams, G.: Proximate composition of American food materials. Washington, D. C., U. S. Dept. Agriculture Circ. No. 549, 1940.

[17] Berry, J. W., Chappell, D. G., and Barnes, R. B.: Improved method of flame photometry. Ind. & Eng. Chem. (Anal. Ed.) **18:** 19, 1946.

[18] Ball, E. G., and Wilson, D. W.: A study of the estimation of chloride in blood and serum. J. Biol. Chem. **79:** 221, 1928.

[19] Gentzkow, C. J.: An accurate method for the determination of blood urea nitrogen by direct nesslerization. J. Biol. Chem. **143:** 531, 1942.

[20] Mudge, G. H., and Vislocky, K.: Electrolyte changes in human striated muscle in acidosis and alkalosis. J. Clin. Investigation **28:** 482, 1949.

[21] Berliner, R. W., and Kennedy, T. J., Jr.: Renal tubular secretion of potassium in the normal dog. Proc. Soc. Exper. Biol. & Med. **67:** 542, 1948.

[22] Mudge, G. H., Ames, A., III, Foulks, J., and Gilman, A.: Effect of drugs on renal secretion of potassium in the dog. Am. J. Physiol. **161:** 151, 1950.

[23] Baldwin, D., Crosley, A. P., Jr., and Talso, P. J.: Influence of various diuretic substances on the renal excretion of electrolytes in the dog. Am. J. Physiol. **155:** 425, 1948.

[24] Berliner, R. W., Kennedy, T. J., Jr., and Hilton, J. G.: Personal communication.

[25] Darrow, D. C.: Retention of electrolyte during recovery from severe dehydration due to diarrhea. J. Pediat. **28:** 515, 1946.

[26] Berger, E. Y., Dunning, M. F., Steele, J. M., Jackenthal, R., and Brodie, B. B.: Estima-

tion of intracellular water in man. Am. J. Physiol. **162:** 318, 1950.

[27] LEVITT, M. F., AND GAUDINO, M.: Measurement of body water compartments. Am. J. Med. **9:** 208, 1950.

[28] YANNET, H., AND DARROW, D. C.: The effect of depletion of extracellular electrolytes on the chemical composition of skeletal muscle, liver, and cardiac muscle. J. Biol. Chem. **134:** 721, 1940.

[29] DARROW, D. C.: Body fluid physiology: The role of potassium in clinical disturbances of body water and electrolyte. New England J. Med. **242:** 978, 1950.

[30] KEITH, N. M., AND WHELAN, M.: A study of the action of ammonium chloride and organic mercurial compounds. J. Clin. Investigation **3:** 149, 1926.

[31] HARRIS, I., RUBIN, E. L., AND LAWRENCE, J. S.: Salyrgan and ammonium chloride as diuretics in cardiac oedema. Acta med. Scandinav. **83:** 23, 1934.

[32] BLUMGART, H. L., GILLIGAN, D. R., LEVY, R. C., BROWN, M. G., AND VOLK, M. C.: Action of diuretics on normal persons. Arch. Int. Med. **54:** 40, 1934.

[33] —, —, AND VOLK, M. C.: Effect of diuretic drugs on the acid-base equilibrium of the blood in patients with cardiac edema. Medical Papers dedicated to Henry A. Christian. Baltimore, Waverly Press, 1936. Pp. 191–203.

[34] GRIGGS, D. E., AND JOHNS, V. J.: Influence of mercurial diuretics on the excretion of sodium, potassium and chlorides. California Med. **69:** 133, 1948.

[35] CRAWFORD, J. H., AND MCINTOSH, J. F.: Observations on the use of novasurol in edema due to heart failure. J. Clin. Investigation **1:** 333, 1925.

[36] ATCHLEY, D. W., AND BENEDICT, E. M.: Serum electrolyte studies in normal and pathological conditions, pneumonia, renal edema, cardiac edema, uremia and diabetic acidosis. J. Clin. Investigation **9:** 265, 1930.

[37] SOLOFF, L. A.: Some clinical aspects of refractory heart failure. Mod. Concepts of Cardiovas. Dis. **19:** 73, 1950.

[38] DARROW, D. C., SCHWARTZ, R., IANUCCI, J. F., AND COVILLE, F.: Relation of serum bicarbonate concentration to muscle composition. J. Clin. Investigation **27:** 198, 1948.

[39] PITTS, R. F., AND SARTORIUS, O. W.: Mechanism of action and therapeutic use of diuretics. J. Pharmacol. & Exper. Therap. **98:** 161, 1950.

[40] ETHRIDGE, C. B., MYERS, D. W., AND FULTON, M. N.: Modifying effect of various inorganic salts on the diuretic action of salyrgan. Arch. Int. Med. **57:** 714, 1936.

[41] EVANS, W. A.: The effect of changes in salt and water metabolism upon salyrgan diuresis. Medical Papers Dedicated to Henry A. Christian. Baltimore, Waverly Press, 1936. Pp. 204–222.

[42] PITTS, R. F., AND DUGGAN, J. J.: The relationship between glomerular filtration rate, proximal tubular absorption of sodium and diuretic efficacy of mercurials. J. Clin. Investigation **29:** 372, 1950.

[43] AXELROD, D. R., CAPPS, J. N., AND PITTS, R. F.: Potentiation of diuretic action of salyrgan by ammonium chloride. Federation Proc. **9:** 6, 1950.

The Pattern of Vascular Reactivity in Experimental Hypertension of Varied Origin

By IRVINE H. PAGE, M.D., AND JAMES MCCUBBIN, M.D.

The response of the arterial pressure to a variety of drugs acting on different parts of the vascular tree has been employed to determine the contrasting mechanisms of chronic hypertension experimentally produced in dogs. The results suggest that the pattern of response depends on the state of the extrinsic regulatory mechanisms of the blood vessels rather than on intrinsic change in vascular musculature. Contrasting with chronic renal hypertension, greatly increased vasomotor function causes increased peripheral resistance in chronic neurogenic hypertension. The hypertension seems to us to be due more to this increase in resistance than to increased cardiac output. The pattern of vascular responsiveness in acute hypertension is different from the chronic and emphasizes the importance of distinguishing between the two phases of hypertension.

THE USE of vasoactive drugs with varied sites of action might elucidate the different mechanisms underlying arterial hypertension. To this end, chronic hypertension was elicited by (1) chronic perinephritis, a renal type, (2) buffer nerve resection and (3) cerebral ischemia, the latter two neurogenic types. In contrast, hypertension of brief duration was produced by infusion of adrenaline, l-noradrenaline, renin and angiotonin, cerebral asphyxia, cerebral compression, perfusion of the brain with histamine and, finally, acute buffer nerve section.

The test drugs were adrenaline and noradrenaline, the one predominantly cardiac and the other peripheral in action; angiotonin and barium chloride, with primarily myotropic action; histamine, with primary action on arterioles and capillaries; veratrum viride, with action on the von Bezold cardiac reflex and the sympathetic system; tetraethylammonium chloride (TEAC), with primarily autonomic ganglionic blocking action; and, in small doses, sodium azide, an enzyme poisoning agent which has a mixed action but chiefly affects the peripheral circulation.[1]

Since there is fairly wide spontaneous variability in different animals and in the same animal at different times[2] it was necessary to use large numbers of dogs and to repeat the tests often. Using the animal as its own control and attempting to establish a trend in a large number of animals has aided in achieving consistency.

It will be evident after the results are examined that a few drugs do, in fact, characterize the mechanism underlying certain types of hypertension by forming a response pattern which may be helpful in the analysis of mechanism in patients. Doubtless many more revealing drugs will be found.

From the Research Division of the Cleveland Clinic Foundation, and the Frank E. Bunts Educational Institute, Cleveland, Ohio.

METHODS

These were described for tests of reactivity by Page and Taylor.[2] Paritol satisfactorily replaced heparin in the tubing to the manometers. We have also used an intratracheal tube. Most of the dogs received a prophylactic dose of penicillin at the conclusion of the experiments. They were kept hydrated by a slow intravenous drip of normal saline throughout the test period.

We would like to emphasize again that, unless arterial pressure is recorded continuously so that the entire curve may be inspected, tests of reactivity are highly precarious. Periodic measurements of arterial pressure at arbitrarily chosen intervals, so frequently done in patients, are inadequate.

NEUROGENIC HYPERTENSIONS

(1*a*) *Chronic Hypertension from Buffer Nerve Section ("Carotid Sinus Hypertension")*

Chronic experimental neurogenic hypertension in dogs is not a constant hypertension, but one associated with wide fluctations in arterial pressure. This variability persists during hours of observation under sodium pentobarbital anesthesia, so that there is opportunity to compare vasoactive drugs at various arterial pressure levels during the same experiment.

Fortunately, marked fluctuation in arterial pressure is usually limited to the first part of the experiment and after one to one and one-half hours a reasonably constant hypertensive level is assumed. This change will occur independently of intravenously given fluids but may often be hastened by small amounts of saline or multiple small doses of noradrenaline.

These dogs are very sensitive to sodium pentobarbital. To our dismay, the customary dose given intravenously to normal dogs (32 mg. per Kg. of body weight) resulted in respiratory depression, vascular collapse and death. The reasons for this increased susceptibility are not apparent.

Extreme hypotension and even cardiac standstill occurred, although the animal was maintained on artificial respiration. The possibility that cerebral anemia as the result of carotid occlusion might have been primarily responsible for increased susceptibility was excluded by section of the nerve of Hering alone, instead of excision of the whole sinus. A safe initial dose was 16 mg. per Kg. sodium pentobarbital intravenously, given slowly, and followed by multiple small doses at 10 to 15 minute intervals until the desired level of anesthesia was reached. Even with this more cautious technic, respiration often ceased during the initial stages of anesthesia while still quite light.

Small doses of pentobarbital given intravenously during the experiment resulted in a sharp and marked drop in blood pressure compared to normal dogs. Depth of anesthesia had no constant or predictable effect on eventual arterial pressure or reactivity. During light anesthesia, a stretch reflex, elicited by manipulation of the needle into which injections were given, caused a severe drop in pressure which was to some extent proportional to the initial height of arterial pressure.

Before hypertension was elicited in the normal control dogs, mean blood pressure levels under pentobarbital anesthesia were borderline or frankly hypertensive in most animals. This is in accord with a previous report[3] on the effect of intraperitoneal sodium pentobarbital on arterial pressure in dogs.

Except in 2 experiments, arterial pressures were significantly higher in the hypertensive animals under pentobarbital anesthesia than during the control tests under pentobarbital. This observation is a cogent argument against the concept that increased arterial pressure in experimental neurogenic hypertension is solely a transient result of excitement and tachycardia.

Adrenaline. Successive small doses of adrenaline, injected intravenously into normal and renal hypertensive dogs, were often accompanied by progressive increase in magnitude of response for the first three or four doses.[2] This was also true in experimental neurogenic hypertensive dogs. The adrenaline responses in table 1 are, therefore, an average of the first four injections in each experiment.

Change in responsiveness after buffer nerve section did not vary in a constant or significant way in any one animal. The average change in the group was a decrease of 6 mm. Hg.

Variation in reactivity at different blood pressure levels during a single experiment was observed repeatedly but changes were of very small magnitude. Thus, adrenaline given at a normotensive level consistently caused a greater response than at markedly hypertensive levels during the same experiment but the difference amounted to only a small fraction of the entire response (fig. 1).

Contrary to the work of others[4] who showed that a depressor response to adrenaline is due indirectly to stimulation of baroreceptors in the carotid sinus and aortic arch, a diphasic response to adrenaline (pressor followed by depressor) was found in 19 experiments after buffer nerve section and in only 5 of the control experiments. The depressor component of this diphasic response was usually prominent at the first injection and then became smaller, often disappearing as repeated injections of adrenaline were given. Pure depressor responses are not unusual in dogs with both carotid sinuses ablated.

In 4 hypertensive dogs exhibiting especially prominent diphasic responses to adrenaline, the sympathetic ganglions from T-1 to T-9 inclusive were removed bilaterally, thus depriving the heart of its sympathetic innervation. In 2 of these animals, the heart was completely denervated by section in the chest of all vagal fibers

TABLE 1.—*Vascular Reactivity in Chronic Neurogenic Hypertension*

	Normotensive Controls			Neurogenic Hypertensives	
	Number of Dogs	Average Response in mm. Hg	Average Response in Per Cent Initial B.P	Average Response in mm. Hg	Average Response in Per Cent Initial B.P.
A. Before and after Production of Hypertension					
Adrenaline	30	+25	15	+18	9
Noradrenaline	30	+33	21	+52	18
Histamine	25	−36	22	−62	35
TEAC	21	−41	26	−95	57

B. Effect of Cardiac Sympathectomy on Neurogenic Hypertension

	Neurogenic Hypertensives		After Cardiac Sympathectomy
		Response in mm. Hg	Response in mm. Hg
Adrenaline	1	+6–34	36
	1	+12–7	22
Noradrenaline	1	+11	57
	1	+19	26

C. Effect of Complete Cardiac Denervation on Neurogenic Hypertension

	Neurogenic Hypertensives		After Complete Cardiac Denervation
Adrenaline	1	+28–21	30
	1	+8–35	66
Noradrenaline	1	+36	61
	1	+45	66
	1	+26	112
	1	+35	61

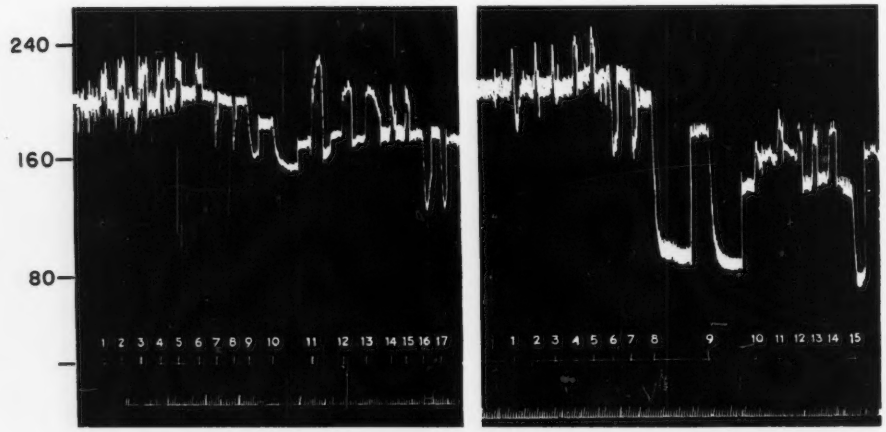


FIG. 1. Typical change in reactivity pattern in chronic neurogenic hypertensive dog following buffer nerve section. (*Left*) *Normal Control.* (1–4) Adrenaline. (5–6) Noradrenaline. (7–8) Histamine. (9–10) Tetraethylammonium. (11–13) Adrenaline. (14–15) Noradrenaline. (16–17) Histamine. (*Right*) *After Producing Chronic Neurogenic Hypertension.* (1–3) Adrenaline. (4–5) Noradrenaline. (6–7) Histamine.(8–9) Tetraethylammonium. (10–12) Adrenaline. (13–14) Noradrenaline. (15) Histamine.

running to the heart, according to the method described by Cannon, Lewis and Britton.[5] Both procedures caused a striking change in responsiveness (table 1). Magnitude of adrenaline response was markedly increased in all 4 animals and in each case the response was purely pressor, the prominent depressor limb of the diphasic response having disappeared.

Noradrenaline. As with adrenaline, change in reactivity after buffer nerve section showed no consistent trend. The average change in reactivity for 30 dogs was a decrease of 1 mm. Hg or, expressed as difference in percentage responses, minus 3 per cent. The responses were always monophasic. Responsiveness, as with adrenaline, tended to be slightly greater at low pressure levels than at high levels, but again, differences were small and not always demonstrable. The 4 previously mentioned hypertensive dogs with complete and partial cardiac denervation were also tested for reactivity to noradrenaline before and after this procedure. Reactivity was greatly increased in 3 animals and moderately increased in the fourth.

Histamine. An increase in reactivity to histamine occurred in 23 of 25 dogs after buffer nerve section. Average change in reactivity was +25 mm. Hg or, expressed as a percentage, +13 per cent. There was no strict over-all correlation between responsiveness and initial mean systolic blood pressure level. In any one experiment however, reactivity was consistently greater at higher arterial pressure levels. In 8 of 25 experiments after buffer nerve section, mean pressure at time of testing was as low as, or lower than, the mean pressure during the control experiment. The increased responsiveness of these dogs was much the same as that of those who were hypertensive.

Tetraethylammonium Chloride. Reactivity to tetraethylammonium was greatly increased in all experiments after section of the buffer nerves. A dose of 5 mg. per Kg. had been given quickly during each of the control experiments. In neurogenic hypertension, the same dose often resulted in an extreme fall in arterial pressure, occasionally followed by vascular collapse and death. For this reason, a dose of 2.5 mg. per Kg. was given in 3 experiments after buffer nerve section and 5 mg. per Kg. in the other 8, one animal dying as a result of the latter dose. The smaller dose of 2.5 mg. per Kg. was almost as effective in producing hypotension in neurogenic hypertensive dogs as the larger one. The over-all average increase was 54 mm. Hg or an increase in percentage response of 30.

The maximal tetraethylammonium-induced fall in pressure following injection of 2.5 mg. per Kg. was greater and reached a lower absolute level than did 5 mg. per Kg. on control experiments. The average mean pressure in the control group after tetraethylammonium was 119 mm. Hg and after buffer nerve section was

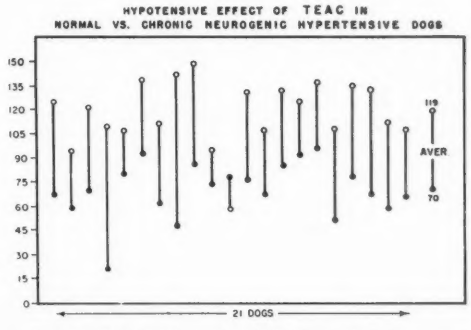


FIG. 2. Points of maximal fall in arterial pressure following tetraethylammonium are represented by ○ for the control determinations and by ● after the same animal was made hypertensive by buffer nerve section.

70 mm. Hg (fig. 2). Moe, Rennick, Capo, and Marshall[6] observed that during acute experiments in dogs under barbiturate anesthesia, tetraethylammonium, given before and after section of the buffer nerves in a dose sufficient to obtain a maximal response, lowered arterial pressure to nearly identical levels on both occasions. Probably the disagreement of our findings with these observations lies in the fact that their experiments were done on dogs with acute neurogenic hypertension while we have been concerned here with the chronic form. These appear to be two separate entities and their confusion may explain much of the contradictory work that has appeared on the etiology of neurogenic hypertension.

No strict correlation between responsiveness

to tetraethylammonium and height of initial mean systolic blood pressure was observed. Rather, tetraethylammonium, like histamine, elicited large responses whether the initial blood pressure was relatively high or low.

Besides the striking increase in depressor response to tetraethylammonium, another important characteristic was demonstrable. Tetraethylammonium given in repeated doses to normal dogs soon elicited a rise in arterial pressure instead of a fall.[7] Usually three doses

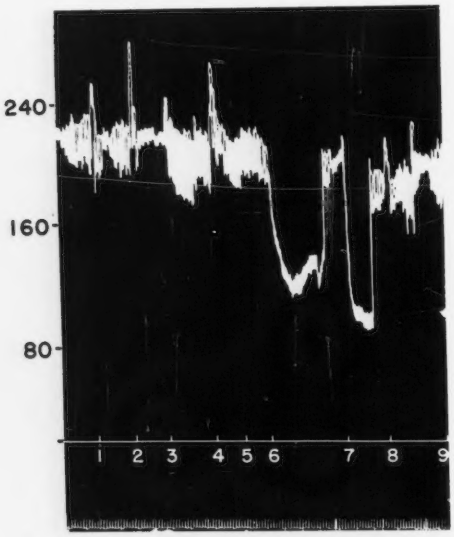


FIG. 3. Responses of a cerebral hypertensive dog under pentobarbital anesthesia (wire placed in medulla, all vessels to brain tied except the right carotid, and finally the right carotid). (1) Adrenaline. (2) Noradrenaline. (3) Barium chloride. (4) Noradrenaline. (5) Angiotonin. (6) Veratrum. (7) Tetraethylammonium, 5 mg. per Kg. (8) Adrenaline. (9) Noradrenaline. (No. 1226).

of 5 mg. per Kg. each sufficed. In contrast, repeated doses of 2.5 mg. per Kg. each in neurogenic hypertensive dogs exhibited little tendency for the depressor response to reverse itself. This was especially well illustrated in dog No. 2262. The initial response was −108 mm. Hg; it was −80 after the twelfth dose of tetraethylammonium. Augmentation of adrenaline, noradrenaline and barium chloride responses, which normally occurs after large doses of tetraethylammonium, was reduced in most cases and absent in some. Initial diphasic or depressor responses to adrenaline became monophasically pressor just as after surgical denervation of the heart.

Angiotonin; Barium Chloride. Responses to these myotropic drugs were not altered significantly. As in normal dogs, frequent repetition of the dose of barium chloride usually led to reduction in blood pressure rise.

Sodium Azide; Veratrum. Both of these depressor drugs, although they have different mechanisms of action, caused greater than normal hypotensive responses in the neurogenic hypertensive dogs. This was a regular occurrence. Increase in responsiveness, however, was not nearly so great as with tetraethylammonium.

(1b) *Chronic Cerebral Irritative and Ischemic Hypertension*

Arterial pressure was elevated to an average of 200 mm. Hg in 4 dogs by tying off most of the blood vessels to the brain, implanting a wire in the floor of the fourth ventricle and treating the head with inductothermy according to the method described by Taylor and Page.[8] These animals had been hypertensive for several months (fig. 3).

The most significant changes in responsiveness in these animals were the great increases in the depressor actions of tetraethylammonium, veratrum and sodium azide (table 2). The responses to other test drugs were unchanged.

(1c) *Hypertension Elicited by Perfusion of Dog's Brain with Histamine*

With the help of Dr. Robert D. Taylor, a few experiments were performed in which histamine was infused into the body of a donor animal connected via the carotid artery and jugular vein to the head of a recipient. The recipient's head was connected to its body only by the nervous system—a preparation described in detail by Taylor and Page.[9] The carotid sinuses were inactivated. No significant difference in response was observed whether the vagus nerves were intact or not. As they have shown, when histamine produces hypotension in the donor animal's body, hypertension occurs in the recipient's body.

During such hypertension, the responses to

noradrenaline and adrenaline almost disappeared but quickly returned on discontinuing the histamine. For example, responses to adrenaline and noradrenaline were 24 and 70 mm. Hg respectively in the recipient's body. Infusion of histamine into the donor's body elicited a sharp, prolonged fall in arterial pressure in the recipient's head and, within a minute, a sharp rise—136 to 262 mm. Hg—in the body. Responses to adrenaline and noradrenaline were then +4 and +6 mm. Hg. The infusion was stopped, the blood pressure fell to 76 and within three minutes response to noradrenaline was +44 and to adrenaline +20 mm. Hg. Even when the elevations in the recipients' arterial pressures were not so great as this, responsiveness was significantly diminished.

Asphyxial Hypertension in Donor Animal. Two experiments were done in which the donor animal was asphyxiated, causing a rise in arterial pressure in the recipient's body (carotid sinuses removed and vagus nerves cut). In one dog, for example, blood pressure rose from 120 to 204 mm. Hg. The response to both adrenaline and noradrenaline was reduced about one half but not abolished.

(1*d*) *Acute Hypertension Due to Artificially Increased Intracranial Pressure*

A 18 gage short bevelled needle was inserted into the cisterna magna and a saline reservoir connected to it. Pressure was measured in a manometer through a sidearm. Reactivity was tested, then the intracranial pressure raised until the animal's arterial pressure reached the desired height. When the level was sufficiently constant, reactivity was again determined (fig. 4).

The results, examples of which are shown in table 2, show that elevation of arterial pressure to average between 212 and 224 mm. Hg did not significantly change responses to adrenaline and noradrenaline. Responses to veratrum and angiotonin were slightly reduced while that to tetraethylammonium was much increased. Tetraethylammonium had little or no augmenting effect on responsiveness to vasoactive agents when the arterial pressure was at high levels. In one experiment the depressor response to sodium azide was nearly doubled.

(1*e*) *Acute Buffer Nerve Section*

After responsiveness had been determined, both carotid sinuses were excluded from the circulation and both vagus nerves cut. In one dog, for example, arterial pressure rose from 160 to 222 mm. Hg. The responses to adrenaline and noradrenaline before were +16 and +70 mm. Hg respectively before section and +6

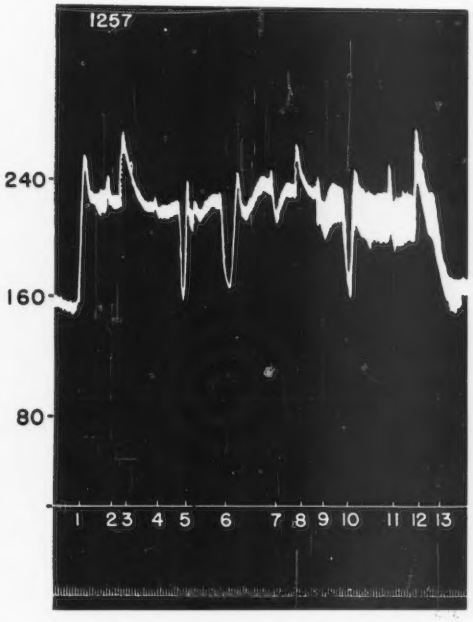


FIG. 4. Hypertension resulting from increased intracranial pressure. 1) Intracisternal pressure increased. (2) Adrenaline. (3) Noradrenaline. (4) Veratrum. (5) Sodium azide. (6) Tetraethylammonium, 5 mg. per kg. (7) Adrenaline. (8) Noradrenaline. (9) Adrenaline. (10) Tetraethylammonium. (11) Adrenaline. (12) Noradrenaline. (13) Pressure off. (No. 1257).

and +30 respectively after. Clearly, with rise in pressure, responsiveness fell sharply. When the blood pressure returned spontaneously to control levels, responsiveness again increased, adrenaline + 32 and noradrenaline +42 mm. Hg.

TEAC responses conformed to no regular pattern. The first one or two injections (2.5 to 5 mg. per Kg.) during the acute hypertension just after buffer nerve section were ordinarily associated with significantly increased vasode-

TABLE 2.—*Examples of Change in Vascular Reactivity with Acute and Chronic Hypertension*

Expt. No.	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	Veratrum	Angiotonin	Renin	TEAC	NaN_3	$BaCl_2$	B.P.
					(1b) Chronic Cerebral Ischemic Hypertension						
1255	30	46	21		−70		20	−84	−96		180
1226	26	56	22	46	−94		8	−110		24	210
1150	24	30	16		−84			−108		36	204
1127	42	32	22		−78					24	140
					(1d) Increased Intracerebral Pressure						
1257	10	30	12	48	−14				−34		144
	10	44	−24		−6			−62	−62		224 Pressure On
	−14	24	20	46				−56			212 Pressure On
	10	64	18	56							124
1262	32	52			−8	26			−42		130
	28	48	30	46	−4	28		−112	−44		184 Pressure On
	14	24				8					212 Pressure On
	18	34				26					176 Pressure On
	12	44									150
					(1f) Total Sympathectomy						
1097	−40	56	−36		−8	24		−28	−72	10	150
1098	−38	92			−6			−14	−72	30	144
1285	−64	44			−16			+12		10	140
								−32			
1276	−20	104			0			−8		10	120
1318	+66	162				28				56	108
1336	+32	102				30				38	140
					(1h) Cardiac Sympathectomy in Normal Animals						
1339	16	80								22	150
1347	24	72			−38					20	124
1350	30	74			−50			−26		16	140
1355	26	64			+16						134
					Cardiac Denervation in Normal Animals						
1338	68	146			6	10				42	110
1342	32	98			6			−36		16	164
1367	30	76			0			−32		24	130
1401	26	86				10				16	126
					(3a) Adrenaline Infusion						
1181	40	68			−26						140
	0	−14 +4		−10	−32						204 Adrenaline
		10			−26				−36		188
	34	70			−14						110
1265	24	48			0				−36		160
	4	38	10	14							168 Adrenaline after 2 hrs. infusion.
	0	14	0	12							216 Adrenaline after 4 hrs. infusion.
	0	−12						42	−26		206 Adrenaline after 5 hrs. infusion.
	8	26	18	36							88

TABLE 2—*Continued*

Expt. No.	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	Veratrum	Angiotonin	Renin	TEAC	NaN_3	$BaCl_2$	B.P.
					(*3a*) *Adrenaline Infusion* (continued)						
1213	24	52			−22				−46		130
	14	30	18	46							180 Adrenaline
	22	46	10	22							170 Adrenaline
	0	18		14	−68			58			186 Adrenaline
	52	86		94				18			62
	16	90									106
1219	36	62							−36		110
	0	6							−34		186 Adrenaline
	12	34	30	62							60
		0						26			212 Adrenaline
	26	56									60
					(*3b*) *Noradrenaline Infusion*						
1153	20	56			−48						144
	38	32	12		−42			46			200 Noradrenaline
	36	84									90
1228	30	50			−44						122
	4				−46				−86		200 Noradrenaline
	44	74							−44		110
	0	0							−74		210 Noradrenaline
	12	36	32	38							104
1236	24	50			−38				−42		116
	0	12		14	−30			42			184 Noradrenaline
	40	66	28	52							100
	0	0						40	−26		192 Noradrenaline
	16	36							−14		196 Noradrenaline
									−32		94
1239	26	44			−50	12			−38		166
		0				0		0	−38		246 Noradrenaline
					−38			0	−46		240 Noradrenaline
									−50		200 Noradrenaline
	22	48	52			28			−44		128
					(*3c*) *Angiotonin Infusion*						
1294	74	140								6	100 C-6 cord destroyed
	76	86								26	188 Angiotonin
1295	18	64			−38				−40	16	148
	20	44			0				−32	0	170 Angiotonin
1296	18	64				34			−48	16	100
	36					0			−34		160 Angiotonin
1297	34	120								34	130
								+34		16	240 Angiotonin
					(*3d*) *Renin Infusion*						
1200	28	66									128
	10	48	14					26			204 Renin

TABLE 2—*Continued*

Expt. No.	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	Veratrum	Angiotonin	Renin	TEAC	NaN_3	$BaCl_2$	B.P.
					(*3d*) *Renin Infusion* (continued)						
1222	−28	46			−42			−66			118
	12	20	10	34	−28						230 Renin
	12	40	12	40							180
1228	12	36	32	38	−44				−44		104
					−66				−78		190 Renin
1202	24	52			−74			−52			174
	0	20									240 Renin
	12	40	24	56							210 Renin
	26	72			−38			8			164

pressor responses compared to normal dogs, but were rarely as large as those seen in chronically neurogenic hypertensive animals. Occasionally, hypotensive effects of initial tetraethylammonium injections were little or no greater than in normal dogs. Although magnitude of vasodepressor responses was great in some experiments, arterial pressure never fell to the low levels seen in the chronic hypertensive phase. On the other hand, the responses showed the same tendency to be persistently depressor and not to reverse after repeated injections. This was uniform but much more apparent in some experiments than in others. In one, for example, tetraethylammonium response was reversed by injection of 5 mg. per Kg. before buffer nerve section but only after 85 mg. per Kg., given in 5 mg. per Kg. doses over a period of two and one-half hours, immediately following section. In this experiment, interestingly enough, arterial pressure averaged 200 to 220 mm. Hg after tetraethylammonium reversal. Administration of benzodioxane at this time was followed by a sustained fall in pressure to normotensive levels and pressor response to tetraethylammonium was abolished.

Reactivity Pattern in Normotensive Animals Subjected to Ablation of Various Parts of the Nervous System

(1*f*) *"Total" Lumbodorsal Sympathectomy*

In a two stage operation, both sympathetic chains were removed from the stellate ganglions above to the sacral below and after two weeks or more, studies of reactivity were done. These animals all showed typical Horner's syndrome, normal arterial pressure with slow heart rate, and all had a tendency to shiver and stretch with fall in blood pressure. As noted by Freeman and Rosenblueth[10] and Bacq, Brouha and Heymans[11] struggle during anesthesia was associated with fall instead of rise in blood pressure.

The results in 6 animals (table 2) showed pure depressor responses to adrenaline in 4 and pure pressor responses in 2. This irregular behavior was not related to the length of time after operation that the test was performed. On the other hand, the pressor response to noradrenaline was consistently augmented. Pressor responses to barium chloride and angiotonin were augmented as was the depressor response to sodium azide. Veratrum exhibited considerably less depressor response than normal. Loss of the hypotensive effect of tetrathylammonium was especially striking.

(1*g*) *Spinal Cord Section at C-6*

Pressor responses to adrenaline, noradrenaline, angiotonin and barium chloride were all significantly increased as was the depressor action of veratrum and azide. Tetraethylammonium caused a pure rise in blood pressure in most and a diphasic response in an occasional dog after spinal cord section

(1*h*) *Cardiac Sympathectomy in Normal and Neurogenic Hypertensive Animals*

Removal of sympathetic ganglions from T-1 to T-9 a week or more before the test increased

the responses to adrenaline and noradrenaline significantly and the depressor effect of histamine slightly. Tetraethylammonium was only slightly less hypotensive than normal (table 2).

If the denervation was completed, responsiveness to all of these drugs increased except for tetraethylammonium, which became very slightly depressor, diphasic or more usually pressor. Veratrum gave only pressor responses while adrenaline, as noted above, often gave a depressor response after complete denervation.

In contrast to chronic neurogenic hypertensives, these dogs were not abnormally sensitive to pentobarbital.

Responses to adrenaline, noradrenaline, barium chloride, angiotonin[12] and renin were not abnormal even when the average pressure was as high as 250 mm. Hg. Tetraethylammonium produced its usual lowering of pressure, and, after repeated injection, augmentation of adrenaline and noradrenaline occurred just as in normal dogs. An example of this

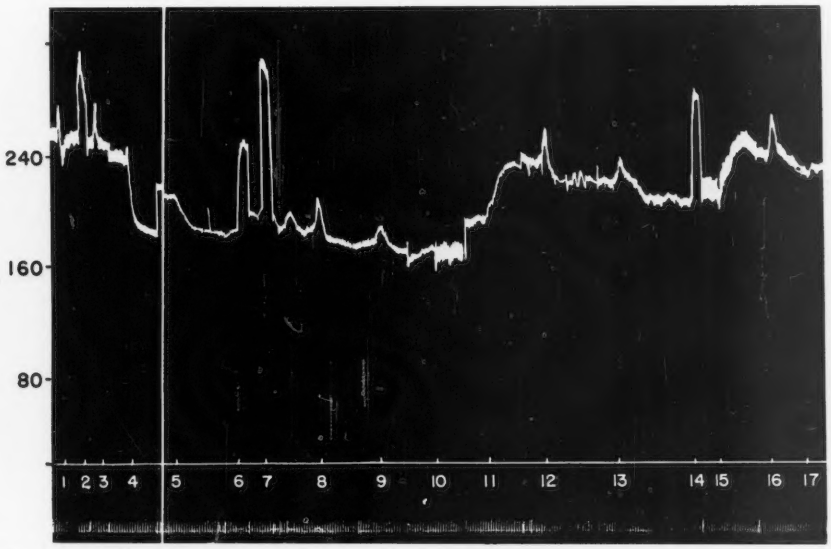


FIG. 5. Responses of a renal hypertensive dog. (Both kidneys wrapped in cellophane. Unanesthetized blood pressure 252 mm. Hg.) (1) Adrenaline. (2) Noradrenaline. (3) Barium chloride. (4–5) Tetraethylammonium, 5 mg. per Kg. (6) Adrenaline. (7) Noradrenaline. (8–9) Tetraethylammonium (10) Barium chloride. (11) Renin. (12–13) Tetraethylammonium. (14) Adrenaline. (15) Renin. (16) Tetraethylammonium. (17) Barium chloride. (No. 958.)

In the neurogenic hypertensive dogs, cardiac sympathectomy caused the diphasic adrenaline response to be replaced by pure pressor action and the noradrenaline responses to be augmented (table 1). Complete denervation of the heart changes this result very little.

CHRONIC EXPERIMENTAL RENAL HYPERTENSION

2a) *Chronic Cellophane Perinephritis*

Eight dogs with arterial pressures ranging from 190 to 250 mm. Hg were tested. In consort of experiment is illustrated in figure 5. Veratrum and sodium azide responses were slightly decreased.

Contrasting with renal hypertensives, normotensive nephrectomized animals (one to two days after operation) showed moderately increased responses to adrenaline and noradrenaline and marked increase to angiotonin and renin. Sodium azide and barium chloride were unchanged. A striking change was the reversal of the depressor effect of tetraethylammonium to a pressor effect when the initial dose was given. This was not invariable but was usual.

(2b) *Malignant Renal Hypertension*

Three dogs developed malignant hypertension as a result of cellophane perinephritis, one having an extremely rapid, fulminating course and dying two weeks after the first rise in pressure. The other 2 gradually entered the malignant phase after renal hypertension was well established; they tolerated the disease well over a period of weeks. The first dog, tested during the period of rapidly rising pressure, showed a striking increase in reactivity to adrenaline, noradrenaline and histamine compared to control determinations before inducing perinephritis. Initial responses to tetraethylammonium were almost entirely pressor, compared with diphasic and mainly depressor control determinations. The second dog, tested during a relatively stable period of its malignant disease, showed essentially normal responses to adrenaline, noradrenaline and barium chloride. Veratrum reduced arterial pressure less than normal. The third dog was terminal and showed much reduced response to noradrenaline and adrenaline but reacted normally to angiotonin and barium chloride. (See fig. 6.)

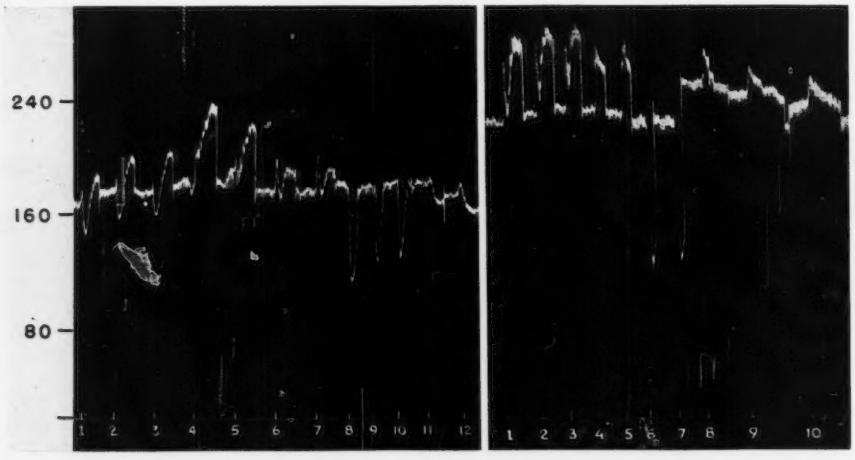


FIG. 6. Vascular responsiveness in the same dog before and after the appearance of malignant renal hypertension.

Left. Normal control (10/12/49)(1–3) Adrenaline. (4–5) Omit nonstandard dose of noradrenaline. (6–7) Noradrenaline. (8) Histamine 0.4 cc. (9–10) Histamine 0.2 cc. (11–12) tetraethylammonium, 5 mg. per Kg. body weight. Kidneys wrapped 10/19/49. *Right.* Malignant hypertension (2/23/50)(1–3). Adrenaline. (4–5) Noradrenaline. (6–7) Histamine 0.2 cc. (8–10) tetraethylammonium, 5 mg. per Kg. body weight.

ACUTE HYPERTENSION CHEMICALLY INDUCED

We think it important to distinguish between hypertension of long standing and that elicited acutely. The following experiments were performed to determine whether the acute variety, produced by substances which may also be the cause of the chronic, would cause changes in reactivity similar to those in animals with chronic experimental hypertension. After control values for the test drugs were determined, hypertension was produced by infusing into normal dogs angiotonin, adrenaline, noradrenaline, etc., and as soon as a stable hypertensive level had been achieved, administering the test drug.

(3a) *Acute Adrenaline Hypertension*

One cc. of a 1:1000 solution of adrenaline in 100 cc. of saline was infused at a rate sufficient to maintain arterial pressure at levels from 180 to 212 mm. Hg. The response to injected adrenaline was abolished and that to noradrenaline almost obliterated. Angiotonin and barium chloride responses were reduced but veratrum

and sodium azide were unchanged. Instead of the usual depressor effect of tetraethylammonium, it was uniformly pressor (table 2). Following its injection, only slight augmentation of response to adrenaline and noradrenaline was observed.

The same responses were seen in dogs in which the spinal cord had been severed at C-6 the day before the experiment. Atropine (1.2 mg.) was given just before the infusion of adrenaline.

a little less pressor than normal while angiotonin itself was ineffective. Tetraethylammonium always produced a rise rather than a fall in blood pressure.

The same pattern of response was observed during angiotonin infusion after section of the spinal cord at C-6.

(3*d*) *Acute Renin Hypertension*

The pressor action of adrenaline and noradrenaline were moderately reduced; in some

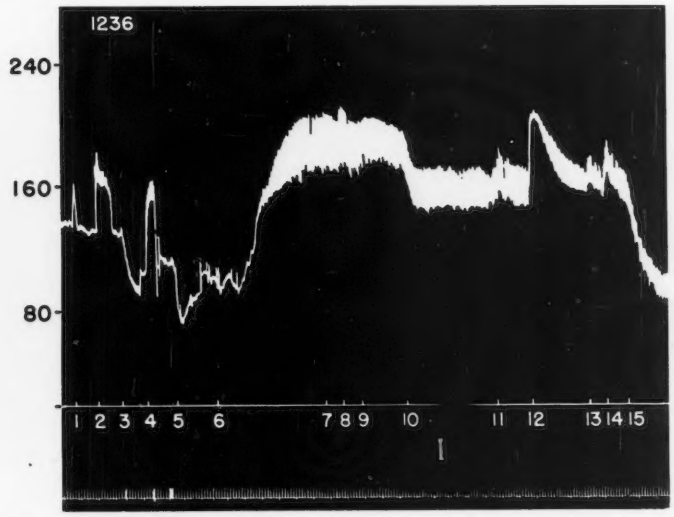


FIG. 7. Noradrenaline hypertension. (1) Adrenaline. (2) Noradrenaline. (3) Veratrum. (4) Noradrenaline. (5) Sodium azide. (6) Infusion noradrenaline. (7) Adrenaline. (8) Noradrenaline. (9) Renin. (10) Veratrum. (11) Noradrenaline. (12) Tetraethylammonium, 5 mg. per Kg. (13) Adrenaline. (14) Noradrenaline. (15) Discontinued noradrenaline infusion. (No. 1236.)

(3*b*) *Acute Noradrenaline Hypertension*

The effects on vascular reactivity were almost identical with those described for adrenaline hypertension (table 2, fig. 7).

(3*c*) *Acute Angiotonin Hypertension*

Infusion of angiotonin at a rate sufficient to elicit hypertension ranging from 210 to 250 mm. Hg caused little change in vascular reactivity (table 2).

Adrenaline and noradrenaline were either unaffected or exhibited slightly reduced responses. Veratrum lost much of its effectiveness in lowering pressure; sodium azide lost less than half of its effectiveness. Barium chloride was possibly

experiments to one half the control values. Veratrum was affected irregularly, sometimes increasing its depressor effect and sometimes decreasing it. The action of sodium azide and barium chloride was unaffected while the pressor effect of angiotonin was lost (table 2).

Tetraethylammonium gave pressor rather than depressor responses.

DISCUSSION

One of the results of this investigation was refutation of the "common sense" belief that smaller responses to pressor vascular stimuli may be expected at high blood pressure levels in well established chronic hypertension. re-

gardless of the mechanism of elevation. It has been assumed that the ability to raise blood pressure is already taxed and little more can be expected. This is far from true. We have many times seen the same rise when the average pressure was 170 as when it was 260 mm. Hg. Injected renin raised the pressure in a renal hypertensive dog from 272 to 340 mm. Hg, which is somewhat more than we would have expected with this dose of renin had the initial pressure been normal. While lower pressures, in comparison with elevated ones, often augment the induced rise from vasoactive substances, the level of arterial pressure is seldom decisive.

The response pattern of dogs anesthetized lightly with pentobarbital does not vary fundamentally from that in unanesthetized dogs. It tends rather to be more regularly reproducible. Pain and discomfort often made the records of even the best trained unanesthetized dogs valueless. Light anesthesia with pentobarbital was used in these experiments and seems to us most desirable.

Whenever possible, the animal acted as its own control by being tested before hypertension was induced. Each dog received from three to eight separate tests over a period of weeks or months. No substitute has been found for careful evaluation of the blood pressure tracing on kymographic records and for repetitive injections of the test drug.

Chronic experimental hypertension should be differentiated from the acute, brief variety induced by infusion of chemical agents or nerve stimulation. With these general observations, we may now consider the specific reaction patterns in dogs with hypertension of varied mechanism.

Comparison of chronic neurogenic with chronic renal hypertension schematized in table 3 shows clearly that the most impressive difference is in the profound hypotensive action of tetraethylammonium in the neurogenic against a normal response in the renal hypertensive dog. In neurogenic hypertension, dose after dose of tetraethylammonium was followed by a precipitous fall in blood pressure with little

TABLE 3.—*Schema of Vascular Response in Hypertensive Dogs*

Pressor Stimulation	Adrenaline	Nor-adrenaline	Angiotonin	Barium Chloride	Veratrum	Sodium azide	TEAC	Avg. B.P.
(1*a*) Chronic—Buffer section	−±	0	−	0	++	++	++++	202
(1*b*) Chronic cerebral ischemic	0	0	0	0	++++	+++	++++	200
(1*c*) Cerebral histaminic hypertension	−−−−	−−−−						242
(1*d*) Increased intracranial pressure	0	0	−		−−	++	++++	224
(1*e*) Acute—Buffer section	−−	−−	−−	0			++	220
(2*a*) Chronic renal experimental	0	0	0	0	−	−	0	250
(3*a*) Infusion adrenaline	−−−−	−−−	−−	−	0	0	Rise	192
(3*b*) Infusion noradrenaline	−−−−	−−−	−−	−	0	0	Rise	210
(3*c*) Infusion angiotonin	0	−	−−−−	−	−−	−	Rise	206
(3*d*) Renin infusion	−−	−−	−−−−	0	0	0	Rise	220
Controls without Hypertension								
Sympathectomy	+++	+++	+	+	−−−	+	−−−	138
Cord section C-6	+++	+++	+++	+++		0	Rise	100

++++ = increase in characteristic action of the drug.
−−−− = decrease in characteristic action of the drug.

Table 3.—*Schema of Vascular Response in Hypertensive Dogs*

or no tendency to exhibit lesser responses as the quantity of drug in the animal's body increased. In normal dogs after two to four doses of 5 mg. per Kg. each, a pressor rather than a depressor response is elicited, believed to be due partially to compensatory liberation of a noradrenaline-like substance from the liver.[7,13] The failure to block autonomic transmission except by heroic doses of tetraethylammonium and the frequent failure to augment significantly other vasoactive drugs in neurogenic hypertensive dogs have no adequate explanation.

Less striking differences are the greater depressor effects of sodium azide and histamine in the neurogenic hypertensive. Azide has a mixed action, but it affects the vascular muscle primarily; we found that destruction of the cord did not alter the response greatly. Histamine acts both on arterioles and capillaries. Mixed veratrum alkaloids (Veriloid*) also lower pressure more effectively in neurogenic hypertensives; their effect is slightly reduced in renal hypertensives. Veratrum acts by stimulating the von Bezold reflex to slow the heart and causes the sympathetic nervous system to decrease peripheral resistance. Its greater effect in the neurogenic hypertensive might be expected because of increased activity of the sympathetic nervous system.

The three myotropic pressor agents, angiotonin, noradrenaline and barium chloride, produced no different effect in chronic hypertension of either renal or nervous origin. Adrenaline had like results except for minor variations concerned with nervous regulatory mechanisms.

In the absence of evidence to the contrary, we conclude that in the stage of compensated chronic hypertension the capacity of the arterial tree for constriction is normal and unchanged, even if the stimulus for the existing hypertension is of such divergent origin as the kidneys or the nervous system. It is only the mechanisms which regulate the caliber of the blood vessels that are disordered. Further experimentation will obviously be necessary to determine whether this hypothesis will be fruitful. If it should prove true, future research would be directed into somewhat different channels than if it were not.

The effect of carbon dioxide inhalation supports this view. Contrary to certain earlier physiologic work, it has now been shown that the effect of carbon dioxide on vascular reactivity is primarily mediated by sympathetic ganglions rather than directly on the vascular muscle itself.[14] The differences between the responses of the neurogenic and renal hypertensive to carbon dioxide seems, in part, to lie in the autonomic nervous system rather than in the blood vessels themselves. In neurogenic hypertension, its enhanced action on the vasoregulatory function of the autonomic system is of prime importance, while in renal hypertension the vasodilator function is no greater than in normal animals.

The vascular response pattern in sympathectomized dogs without hypertension also lends some support. Adrenaline is irregular in its action, sometimes producing a fall in pressure and, more often, an augmented rise. Noradrenaline is greatly augmented; angiotonin, barium chloride and sodium azide are only moderately augmented. Because of the interrupted nerve pathways, the actions of both veratrum and tetraethylammonium are sharply reduced. Thus, drugs dependent for their action on the sympathetic nervous system lose their characteristic effects after sympathectomy. On the contrary, drugs dependent on the musculature of the vessels and heart exhibit their characteristic actions in increased degree because of the loss of inhibitory sympathetic functions.

According to Olsen, Schroeder, and Menhard,[15] renal hypertensive rats exhibit greater response to adrenaline and noradrenaline and much less to isoamylamine than normal. Phenylethylamine and tyramine gave the same responses in both. The observations obviously differ from ours in dogs and do not support the hypothesis that the mechanism of constriction in blood vessels is unchanged in chronic hypertension regardless of its origin.

After the establishment of chronic neurogenic hypertension, the response to the usual pressor dose of adrenaline is pressor, diphasic

*Courtesy of Dr. Philip Bates, Riker Chemical Co., Los Angeles, Calif.

or frankly depressor. We have found the latter two types of response so frequently, it seemed of value to learn more of their mechanism. Fortunately, it was soon found that cardiac sympathectomy completely abolished diphasic and depressor responses, leaving the pure pressor.

We believe these results may be explained, on the basis of Ahlquist's work,[16] as due to failure of the reflex neurogenic vasodilatation to occur after cardiac sympathectomy during the acute pressor action of adrenaline before the adrenaline acts directly on the vascular musculature. Failure of this reflex would allow arterial pressure to increase progressively rather than rise initially and then fall.

Thomas and McLean[17] postulated that if neurogenic peripheral vasoconstriction is great in experimental neurogenic hypertension, further stimulation by adrenaline and angiotonin would be less effective than normal. In chronic experiments on 3 unanesthetized dogs, these drugs were injected before and after buffer nerve section with generally similar responses in both duration and magnitude, allowing the conclusion that, while neurogenic vasoconstrictor activity may be increased in neurogenic hypertension, it is not sufficiently great to interfere with the action of these drugs. Our experiments, on a larger number of dogs, confirm these observations.

Much current evidence[17] suggests that peripheral resistance in experimental neurogenic hypertension is little, if at all, increased. Our results with tetraethylammonium and the recent work of Moss and Wakerlin[18] and Grimson,[19] however, seem to leave little doubt that resistance is greatly increased. The interpretation of the increased and consistent vasodepressor effect of tetraethylammonium is complicated. Moe and associates[6] showed that the drug did not reduce cardiac output in spite of cardiac slowing. A dose of tetraethylammonium sufficient to produce a maximal fall in blood pressure, according to their view, reflects the amount of neurogenic peripheral resistance. Other evidence[7, 13] suggests that this is an oversimplification because the injection of tetraethylammonium elicits a compensatory outpouring of a noradrenaline-like pressor substance which tends to raise blood pressure. Thus, repeated injections of tetraethylammonium soon cause a rise rather than fall in arterial pressure. It is possible that the first dose of tetraethylammonium, if by chance it is correctly chosen, could give almost complete blockade without concurrently stimulating compensatory mechanisms. With these reservations in mind, it is evident from our data that fall in blood pressure in chronic neurogenic hypertensives is still far greater than normal, indicating a high degree of neurogenic vasoconstriction. In this, the chronic hypertensive seems to differ from the acutely debuffered animal in which only moderately increased hypotensive effect of tetraethylammonium is observed. It is important to bear in mind that the pattern of vascular response in acute and chronic hypertensives is often different.

The experiments in which hypertension was produced acutely have certain resemblances among themselves, no matter how the hypertension was produced. The responses during adrenaline or noradrenaline infusion were identical. Particularly striking was the rise in arterial pressure on the initial injection of tetraethylammonium. This we believe to be due to the release of compensatory nervous mechanisms which tend to brake the pressor action of the infused drug. The mechanism of such drug augmentation has been described by Page and Taylor.[13] The same rise in blood pressure after tetraethylammonium was noted when angiotonin and renin were infused, which was to be expected on this basis. The self-inhibition of all of these drugs when administered in large quantities by infusion was striking.

The chronic hypertensive does not respond to the test drugs as though the response were patterned by a large endogenous source of pressor substance. For example, in acute renal hypertension produced by renin infusion, the response to angiotonin and to renin disappears, but in chronic renal hypertensives rises of 60 to 90 mm. Hg may be noted in response to renin in animals whose blood pressure is already in the 250 to 280 mm. Hg range. On the other hand, the responses during infusion of angiotonin are much like those of chronic renal hypertension except for the fact that tetra-

ethylammonium raises pressure in the former and lowers it sharply in the latter.

The depressor action of azide is essentially unchanged regardless of what chemical was infused to produce the acute hypertension. This again suggests that as long as the muscle receptors are not saturated as a result of the continuous infusion of the drug, they are able to respond normally, no matter how the blood pressure was raised. The responses to barium chloride are also consistent with this view.

The response to veratrum (Veriloid) was little changed during acute hypertension except in hypertension due to angiotonin and increased intracranial pressure where it was somewhat reduced. That veratrum could still initiate the von Bezold reflex and decrease sympathetic vasoconstriction during periods of acute hypertension is surprising and emphasizes the remarkable ability of the body to hold protective mechanisms in reserve, even under adverse hemodynamic conditions.

Although the results of this investigation may find application in the analysis of reactivity of hypertensive patients, we shall not at present discuss the problem, but certain special cases may be mentioned in which the physiologic mechanisms receive some clarification. Mayock and Rose[20] pointed out that before the removal of a functioning pheochromocytoma, patients are insensitive to adrenaline. We have observed insensitivity to the infused drug when acute hypertension has been established which is not extended to other, chemically unrelated, pressor or depressor substances.

The profound depression of blood pressure after discontinuing infusions of adrenaline and noradrenaline has been noted by many observers and called "adrenaline shock." Our animal experiments agree with those of Green and associates[21] on patients in the rough correlation between the height of the initial pressure and the depth of the postinfusion depression. As estimated by this, vasodilator capacity in hypertensive animals is certainly not impaired.

From a small experience with dogs in the malignant phase of hypertension, sensitivity increases early to be followed by progressively waning response as death approaches. Myotropic substances such as angiotonin and barium chloride were augmented relatively more in the early phase and less in the terminal phase than the other test drugs.

Summary

1. The response to vasoactive drugs of chronically hypertensive dogs is not prescribed by the height of the arterial pressure or light pentobarbital anesthesia.

2. Dogs with chronic neurogenic hypertension from buffer nerve section are highly sensitive to pentobarbital and tetraethylammonium and less sensitive to sodium azide, veratrum and histamine. Adrenaline, noradrenaline and barium chloride responses are unchanged from normal. The same general pattern is seen in chronic cerebral irritative and ischemic hypertension and in acute hypertension due to increased intracranial pressure and to section of the buffer nerves. The patterns in cerebral histaminic hypertension, and after total lumbodorsal sympathectomy, spinal cord section at C-6, cardiac sympathectomy and cardiac denervation, are entirely different.

3. Chronic renal hypertensive dogs respond normally to the test drugs. In early malignant hypertension, responses are increased, to be reduced in the terminal phase. Pressor responses to tetraethylammonium were noted on initial administration, much as after nephrectomy.

4. Acute hypertension elicited by infusions of adrenaline, noradrenaline, renin and angiotonin, are alike in the quick loss of response to test doses of the infused drug and the uniform pressor action of tetraethylammonium. The pattern of reactivity differs from that in chronic hypertension.

5. The biphasic and depressor adrenaline responses in neurogenic hypertensives become pressor after cardiac sympathectomy or denervation.

6. Neurogenic hypertensive dogs are not only highly sensitive to tetraethylammonium, but little, if any, autonomic blockade can be produced by its repeated administration.

Conclusions

The pattern of pressure responsiveness to vasoactive drugs in contrasting forms of chronic

hypertension depends on the state of the extrinsic regulatory mechanisms of the blood vessels and not on intrinsic changes in vascular musculature. The response patterns characterize the mechanisms which underlie chronic experimental hypertension.

Increased vasomotor function increases peripheral resistance in chronic neurogenic hypertension. The increased peripheral resistance in renal hypertension is of a different origin.

Acute hypertension elicited by infusion of a variety of pressor agents differs from chronic hypertension as measured by the pattern of vascular responsiveness. It is unlikely that these patterns can be used as evidence for or against the participation of specific pressor substances in the production of chronic hypertension. They compare quite accurately with acute, transient hypertension in man and provide a physiologic basis for some of the responses observed clinically.

REFERENCES

[1] Graham, J. D. P.: Actions of sodium azide. Brit. J. Pharmacol. **4:** 1, 1949.

[2] Page, I. H., and Taylor, R. D.: Variations of vascular reactivity in normal and hypertensive dogs. Am. J. Physiol. **156:** 412, 1949.

[3] Corcoran, A. C., and Page, I. H.: Effects of anesthetic dosage of pentobarbital sodium on renal function and blood pressure in dogs. Am. J. Physiol. **140:** 234, 1943.

[4] Heymans, C., Bouckaert, J. J., and Regniers, P.: Le sinus carotidien et le zone homologue cardioaortique. Paris, G. Doin et Cie, 1933.

[5] Cannon, W. B., Lewis, J. T., and Britton, S. W.: Studies on conditions of activity in endocrine glands. 17. A lasting preparation of the denervated heart for detecting internal secretion, with evidence for accessory accelerator fibers from the thoracic sympathetic chain. Am. J. Physiol. **77:** 326, 1926.

[6] Moe, G. K., Rennick, B. R., Capo, L. R., and Marshall, M. R.: Tetraethylammonium as an aid in the study of cardiovascular reflexes. Am. J. Physiol. **157:** 158, 1949.

[7] Page, I. H.: Mechanism of the vascular action of tetraethylammonium chloride. Am. J. Physiol. **158:** 403, 1949.

[8] Taylor, R. D., and Page, I. H.: Peripheral vasomotor effects of adrenaline and noradrenaline acting upon the isolated perfused central nervous system. Circulation. In Press.

[9] —, and —: Production of prolonged arterial hypertension in dogs by chronic stimulation of the nervous system. Exploration of the mechanism of hypertension accompanying increased intracranial pressure. Circulation. In Press.

[10] Freeman, N. E., and Rosenblueth, A.: Reflex stimulation and inhibition of vasodilators in sympathectomized animals. Am. J. Physiol. **98:** 454, 1931.

[11] Bacq, Z., Brouha, L., and Heymans, C.: Recherches sur la physiologie et la pharmacologie du système nerveux autonome. Reflexes vasomoteurs d'origine sino-carotidienne et réactions pharmacologiques chez le chat et le chien sympathectomisés. Arch. Internat. de pharmacodyn. et de thérap. **48:** 429, 1934.

[12] Page, I. H.: The pressor response of normal and hypertensive dogs to renin and angiotonin. Am. J. Physiol. **134:** 789, 1941.

[13] —, and Taylor, R. D.: Augmentation of vasoactive substances by tetraethylammonium chloride. Circulation **1:** 1233, 1950.

[14] —, and Olmsted, F.: The influence of respiratory gas mixtures on arterial pressure and vascular reactivity in "normal" and hypertensive dogs. Circulation. In Press. **3:** 801, 1951.

[15] Olsen, N. S., Schroeder, H. A., and Menhard, E. M.: Effect of certain amines on the blood pressure of normotensive and hypertensive rats. Proc. Soc. Exper. Biol. & Med. **74:** 581, 1950.

[16] Ahlquist, R. P.: Influence of acute epinephrine hypertension on calculated resistance of canine femoral vascular bed. Am. J. Physiol. **159:** 471, 1949.

[17] Thomas, C. B., and McLean, R. L.: The effect of intravenous injection of epinephrine and angiotonin before and after the production of neurogenic hypertension. Bull. Johns Hopkins Hosp. **75:** 319, 1944.

[18] Moss, W. G., and Wakerlin, G. E.: Role of the nervous system in experimental renal hypertension. Am. J. Physiol. **161:** 435, 1950.

[19] Grimson, K.: Role of the sympathetic nervous system in hypertension as revealed by action of sympatholytic and depressor drugs. In Factors Regulating Blood Pressure. Transactions of the Josiah Macy Conference, May 5 and 6, 1949. New York, N. Y.

[20] Mayock, R. L., and Rose, E.: Insensitivity to adrenaline in a patient with a functioning tumor of the adrenal medulla. Am. J. M. Sc. **213:** 324, 1947.

[21] Green, D. M., Johnson, A. D., Lobb, A., and Cusik, G.: The effects of adrenaline in normal and hypertensive patients in relation to the mechanism of sustained pressure elevation. J. Lab. & Clin. Med. **33:** 332, 1948.

Studies on Congestive Circulatory Failure

IV. The Effect of Various Diuretics on the Excretion of Water and Chlorides

By HENRY A. SCHROEDER, M.D.

Chloride balances were determined in 40 patients suffering from congestive circulatory failure and 6 with normal cardiac function, while diuretics were administered. The effect of digitalis was to increase the renal excretion of chlorides and water about equally; this action was significant in only one-third of the cases. Mercurial diuretics primarily increased the urinary concentration of chlorides in every case except when plasma chlorides were low. A xanthine (theobromine) appeared to exert a greater action on the excretion of water than on chlorides.

PREVIOUS communications have indicated the relations in congestive circulatory failure between dietary intake of sodium chloride and the formation of edema,[1] and between the excretion of urinary chlorides and changes in the amount of fluids retained in the body.[2] That the kidneys of cardiac patients cannot excrete sodium chloride efficiently was also shown.[3] It is believed that the disturbances leading to the retention of salt and water, while primarily of cardiac origin, involve some other mechanism, set in motion by a damaged heart and mediated through the kidneys. The derangement of that mechanism causes most of the distressing symptoms associated with congestive failure. When that mechanism can be influenced properly, chronic cardiac patients can be made relatively comfortable for long periods, as long, probably, as the primary cardiac disturbance does not progress too rapidly, or accidents do not occur.

During the course of these experiments, it became necessary to study the effects on fluid balance of various common diuretic drugs. Certain of these observations are worth recording, in that they are suggestive of modes by which these diuretic agents act. Data will be presented to show that mercurial diuretics act principally upon chloride excretion, a xanthine appears to act upon water, while digitalis promotes the excretion of both.

From the Hospital of the Rockefeller Institute, New York, New York, and the Department of Internal Medicine, Washington University School of Medicine and Barnes Hospital, Saint Louis, Missouri.

METHODS

The drugs used were mercurial diuretics (Mercupurin, Salyrgan and Salyrgan-theophylline), a xanthine diuretic (Theocalcin or calcium theobromine and calcium salicylate), and digitalis (whole powdered leaf, New York Heart Association); other measures were employed in several experiments. Forty patients suffering from congestive circulatory failure were studied, most of them exhibiting severe "right sided" heart failure. The methods of study were described in a previous report.[1, 2] In addition, 5 patients suffering from arterial hypertension with normal renal and cardiac function, and one from bronchial asthma were observed. Daily measurements were made of chlorides in the urine, the urinary volume and the body weight, while the intake of calories, salt, and fluids was controlled.

RESULTS

I. *The Effect of Digitalis on the Urinary Excretion of Chlorides and Water, and on Body Weight*

After control periods of several days to several weeks, digitalis was given by mouth to 15 patients in doses calculated to be sufficient to improve the circulation. Six exhibited auricular fibrillation, but in only 2 were there large pulse deficits with rapid ventricular rates. Four were extremely ill, and little effect upon the urinary excretion of chlorides or of water was noticed. Six others were in less severe stages of their disease, but the effects were slight or absent. In the remainder, improvement occurred, which was sometimes accompanied by changes in the urinary output of chlorides (table 1). The *concentration* of chlorides, however, did not usually increase in proportion to

TABLE 1.—*Effect of Digitalis on the Urinary Excretion of Chlorides and Water*

Case No. & Date	Intake		Days of Observation	Dose of Digitalis	Output in Urine			Change in Output	Change in Body Weight	Theoretic* Change Calc. from Cl Output	Remarks†
	NaCl	Fluids			Cl as NaCl		Vol.				
	Gm.	*cc.*		*Total Gm*	*Gm./day*	*Gm./L.*	*cc./day*	*cc.*	*Total Kg.*	*Kg.*	
32—J. V. ♀											
10/16/40	1.0	2000	5		.65	.40	1624		+0.3		A. H. D.
10/21/40	1.0	2000	5	2.0	.34	.24	1438	−930	+1.4		
22—C. M. ♂											
1/20/38	1.0	600	3		.11	.59	187		+0.8		R. H. D.
1/23/38	1.0	600	3	1.4	.04	.15	260	219	−0.1		Died
21—E. H. ♀											
6/24/38	1.0	1500	4		.49	.98	500		+1.5		A. H. D.
6/28/38	1.0	1500	2	1.4	.50	.91	545	90	0.0		Aur. Fib.
12—A. J. ♂											
4/4/38	1.0	1200	7		.36	.43	834		+0.9		A. H. D.
4/11/38	1.0	1200	7	2.4	.89	.98	909	520	−1.6		
30—J. K. ♀											
12/4/39	1.0	1500	3		.08	.16	482		+0.8		R. H. D.
12/7/39	1.0	1500	5	2.2	.18	.45	402	−400	+0.4		Died
16—H. M. ♀											
8/25/40	1.0	1500	4		1.62	1.00	1617		+1.0		Aur. Fib.
8/29/40	1.0	1500	6	1.8	1.83	1.10	1657	240	−1.3	−.9	R. H. D.
1/13/38	1.0	1500	5		1.24	1.03	1196		−0.5		
1/19/38	1.0	1500	9	2.8	1.96	1.45	1349	1377	−1.0	−1.5	
40—G. A. ♂											
11/24/37	2.0	2000	8		.36	.19	1853		−1.3		H. H. D.
12/2/37	2.0	2000	8	2.2	1.29	.97	1332	−4168	−0.1		Died
41—L. C. ♂											
3/12/41	1.0	1800±	5		.29	.19	1536		0.0		A. H. D.
3/17/41	1.0	1800±	5	1.5	.25	.17	1512	−120	−0.8		Died
35—N. J. ♂											
11/30/39	1.0	1500	7		1.03	.96	1078		0.0		A. H. D.
12/7/39	1.0	1500	3	2.2	2.14	1.31	1633	1665	−1.8	−.6	Aur. Fib.
13—I. Y. ♂											
10/13/37	2.0	2500	10		.51	.45	1125		−0.4		A. H. D.
10/23/37	2.0	2500	10	2.7	1.48	.89	1660	5350	−0.8		
6—J. B. ♂											
9/6/39	1.0	1500	5		.06	.06	1076		+0.5		A. H. D.
9/11/39	1.0	1500	5	1.8	.12	.08	1546	2350	−1.9		Aur. Fib.
39—C. M. ♂											
4/21/40	4.0	1500	5		1.29	1.65	780		+2.7		A. H. D.
4/26/40	4.0	1500	5	1.8	3.60	3.19	1134	1770	+0.5		Died
19—M. J. ♀											
2/20/38	0.5	1000	9		.49	.46	1060		0.0		A. H. D.
2/29/38	0.5	1000	5	2.0	.13	.14	904	−780	+0.2		Aur. Fib.
43—J. P. ♂											
8/3/39	1.0	1000	5		.10	.09	880		+0.7		A. H. D.
8/8/39	1.0	1000	5	1.2	.20	.34	678	−1010	0.0		Aur. Fib.
42—A. G. ♂											
12/27 39	1.0	1800	7		.54	.56	967		−0.1		A. H. D.
1/3/40	1.0	1800	7	2.6	.64	.56	1139	1204	−3.9		

* This figure was calculated from those cases in which the urinary output of chlorides exceeded the intake. It represents the weight of a fluid containing 100 mEq. of sodium chloride per liter.

† A. H. D. = arteriosclerotic heart disease
R. H. D. = rheumatic heart disease
H. H. D. = hypertensive heart disease
Aur. Fib. = auricular fibrillation
The case numbers are the same as those in the preceding studies.[1,2]

the total output, suggesting that the primary effect of digitalis, when it acted at all, was to increase the excretion of water and salt together. Improvement in the ability of the kidneys to form glomerular filtrate would account for the results.

Analysis of the data indicated that the effect upon salt and water excretion was variable. The average excretion of water was actually diminished (100 cc. or more per day) during administration of digitalis in 4 cases, was relatively unchanged in 6, and increased in 6. The average excretion of chlorides was diminished in 4, relatively unchanged in 6, and increased significantly in 6. Curves of body weight were altered in a favorable direction in 13, but weight (1 Kg. or more) was lost in only 5 cases; 3 of these exhibited auricular fibrillation. In only 3 was an excellent water diuresis established, with the average output increasing by more than 400 cc. per day. The average urinary concentration of chlorides increased considerably in 6, slightly in 2, remained unchanged in 5, decreased slightly in one and markedly in 2.

The average pulse rate was decreased more than 10 beats per minute in 5 subjects with normal sinus rhythm and was unchanged in 4. When auricular fibrillation with a slow ventricular rate was present, it was decreased in one of 4 patients.

II. *The Effect of Theocalcin on Urinary Chlorides and Water, and on Body Weight*

Thirteen patients were studied in 17 experiments. The use of Theocalcin in doses of 3.0 to 4.5 Gm. per day by mouth occasioned usually a marked or moderate increase in the renal excretion of water, often accompanied by chlorides. The concentration of chlorides in the urine was increased proportionately less than was the amount of water, although the total quantity often rose. Sometimes diuresis following the administration of Theocalcin exceeded considerably that caused by other diuretic agents. Occasionally no effect whatsoever was noticed, and in 6, a water diuresis was initiated which was accompanied by little change in a previously low chloride output. In general, the use of this agent appeared to be more effective in controlling edema than was digitalis, especially when normal cardiac rhythm was present (table 2).

The average urinary excretion of chlorides was increased in 12 instances, relatively unchanged in 4, and depressed in one. Average volume of urine was increased more than 100 cc. per day in 14, more than 400 in 10, and more than 700 in 2. It was relatively unchanged in 3. Weight of 1 Kg. or more was lost in 10. The average urinary concentration of chlorides increased considerably in 4, slightly in 4, remained unchanged in 5, fell slightly in 3, and considerably in one. In 7 water diuresis was not accompanied by an increase in chloride concentration.

In 5 patients the effectiveness of digitalis and Theocalcin in causing diuresis was compared. In 4 of these patients Theocalcin produced greater changes in the output of chlorides and water, resulting in greater loss of weight. In the other digitalis was somewhat more effective. There did not appear to be much difference between the effect of the two agents upon chloride excretion, when the largest volumes of urine in any one day were compared (fig. 1), although Theocalcin may have exerted less action upon concentration of chlorides. The pulse rate increased 10 beats per minute or more in 11 of the 13 patients when Theocalcin was administered. Sometimes it increased markedly. This was also true in 2 patients suffering from complete heart block, whose symptoms of syncope were relieved. The pulse rate increased to the same extent both when rhythm was normal or auricular fibrillation had been controlled by digitalis.

III. *The Action of Mercurial Diuretics*

It is now becoming understood that the principal effect of mercurial diuretics is to inhibit the reabsorption of salt by the renal tubules, thereby causing diuresis of water and salts.[3] It is not generally appreciated, however, how much salt the urine may contain. Thirty-eight injections of Salyrgan, six of Salyrgran-theophylline, and sixty-three of Mercupurin were given intravenously to 30 patients with congestive failure and to 6 normal or hypertensive subjects with normal cardiac function. Re-

TABLE 2.—*Effect of Theocalcin on Urinary Excretion of Chlorides and Water*

Case No. & Date	Intake		Days of Observation	Dose Per Day	Output in Urine			Change in Output	Change in Body Weight	Theoretic Change		Remarks
	NaCl	Fluids			Cl as NaCl		Vol.			Calc. from Cl output	Calc. from Water*	
	Gm.	*cc.*		*Gm.*	*Gm./day*	*Gm./L.*	*cc./day*	*cc.*	*Total Kg.*	*Kg.*	*Kg.*	
12—A. J. ♂												
5/7/38	0.5	2500	5	4.5	5.31	1.78	2976	8255	−4.4	−4.1	−8.3	On digitalis
5/12/38	0.5	2000	4	0	2.92	2.25	1325		−0.2	−1.6		A. H. D.
5/16/38	0.5	1800	4	4.5	4.52	2.29	1927	2408	−1.6	−3.1	−2.4	
8—B. H. ♂												
4/20/39	1.0	1200	7		.15	.14	1017		+1.2			A. H. D.
4/27/39	1.0	1200	7	4.5	.03	.03	1063	322	+0.8		−.3	
32—J. V. ♀												
9/20/41	1.0	2000	12		.04	.02	1667		+1.2			A. H. D.
10/11/41	1.0	2000	4	3.0	1.83	1.08	1692	100	−1.1	−.57	−.1	
10/28/40	1.0	1500	3		.38	.30	1251		+0.1			On digitalis
11/1/40	1.0	1500	12	3.0	.81	.48	1703	5424	−3.6		−5.4	
49—S. D. ♂												
5/20/41	1.0	2000	7		1.24	1.02	1213		+0.4			H. H. D.
5/27/41	1.0	2000	7	3.0	2.22	1.38	1614	2807	−1.3	−1.5	−2.8	
6/3/41	1.0	2000	5		.59	.50	1185		+0.6			
36—M. H. ♀												
5/27/41	1.0	1500	3		.04	.06	636		+0.1			R. H. D.
5/30/41	1.0	1500	5	3.0	.24	.22	1092	2280	+0.1		−2.3	
25—W. D. ♂												
5/13/41	1.0	1500	5		.05	.06	830		0.0			R. H. D.
5/18/41	1.0	1500	5	4.5	.13	.14	932	510	0.0		−.5	Aur. Fib.
3/29/41	1.0	1500	7		.83	.82	1010		−0.2			On digitalis
4/5/41	1.0	1500	14	4.5	1.01	.94	1075	910	−2.3		−.9	
	1.0	1500	7		.25	.36	687		+1.8			
38—P. F. ♂												
4/2/41	1.0	2000	5		.06	.09	668		+0.7			R. H. D.
4/7/41	1.0	1200	4	4.5	.08	.07	1203	2140	−2.9		−2.1	
9—H P. ♂.												
4/18/39	1.0	1500	5		.72	.74	976		−2.8			A. H. D.
4/23/39	1.0	1500	5	4.5	.67	.46	1462	2430	−2.7		−2.4	
44—J. S. ♂												
1/19/	1.0	2000	6	4.5	.77	.51	1518	1410	−0.6		−1.4	A. H. D.
1/25/	1.0	2000	4		.57	.45	1283		+0.3			
7—P. O. ♂												
4/14/39	1.0	1400	7		.16	.17	933		+1.1			A. H. D.
4/21/39	1.0	1400	10	4.5	.75	.65	1147	2140	−1.6		−2.1	Heart Block
5/1/39	1.0	1400	7		.21	.30	697		−0.4			
5/13/39	1.0	1400	7	4.5	.50	.35	1424	5089	−3.4		−5.1	
41—L. C. ♂												
2/7/41	1.0	1800±	14		.30	.22	1339		+0.1			A. H. D.
2/21/41	1.0	1800±	7	3.0	.84	.57	1468	903	−0.1		−.9	
35—N. J. ♂												
1/26/40	1.0	2500	12		.85	.46	1865		−2.6			A. H. D.
2/7/40	1.0	2500	14	3.0	1.68	.69	2450	8190	−6.8	−1.6	−8.2	
42—A. G. ♂												
2/14/40	1.0	1500	7		.08	.10	787		0.0			A. H. D.
2/21/40	1.0	1500	8	3.0	.10	.08	1247	3680	−0.3		−3.7	
2/29/40	1.0	1500	7		.24	.20	1215		0.0			

Notations same as in table 1.

* This figure was calculated from the weight of the total increase of urinary volume as compared to the control period.

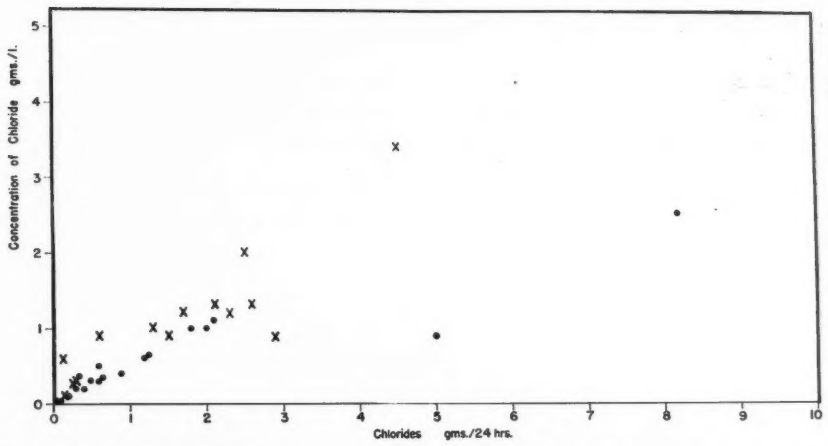


FIG. 1. A relation between the concentration of chloride and the total amount excreted in the largest single volume of urine produced after the use of digitalis (X) and Theocalcin (●). At the lower concentrations it would appear that digitalis sometimes exerts slightly more effect on the concentration of chloride in the urine than does Theocalcin. At the higher concentrations Theocalcin appears to affect principally the excretion of water.

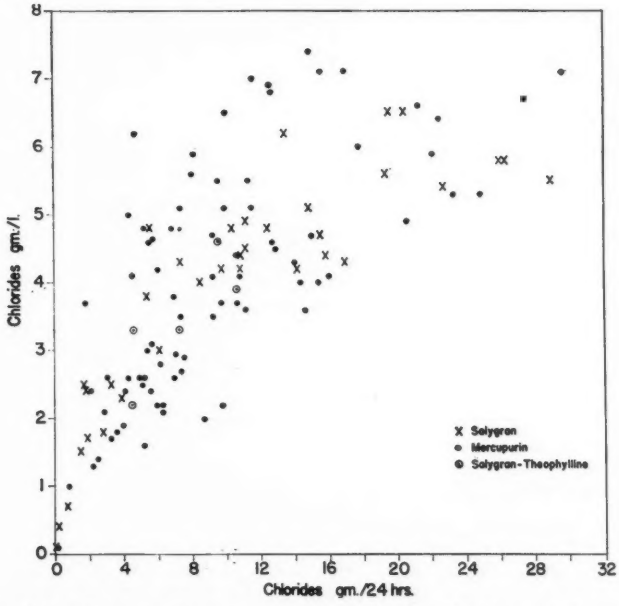


FIG. 2. The relation between the concentration of urinary chlorides and the total amount excreted during the 24 hours following injection of a mercurial diuretic. Note the large amounts of chlorides (as NaCl) which are sometimes excreted. As the amount excreted increases, there appears a tendency for the concentration to become limited; as it falls, the concentration becomes relatively greater. At 5.85 Gm. on the ordinate is the normal value (100 mEq. per L.) of plasma or edema fluid; points above this level indicate occasions when actual depletion of body chlorides occurred.

peated doses were given in 22 cases, and a number of observations made under different circumstances.

(*a*) *Principal Effects.* The usual finding was a marked increase in the concentration of chloride in the urine, indicating that the main action was to cause diuresis of salt, with which some water was removed (fig. 2). When large doses were given (4 to 6 cc.), a maximum concentration appeared to be reached, which was never over 8.0 Gm. (136.8 mEq.) per liter. When minimal doses were given, little change in urinary volume occurred, although the amount and concentration of chloride invariably increased except in one circumstance. When plasma chlorides were low, little or no effect upon urinary salt or water was produced (fig. 3). No exact correlation could be made between the quantity of chloride in the urine and the size of a dose varying from 2.0 to 6.0 cc., although there was a slight tendency for larger doses to be more effective. In 20 instances concentrations in the urine were more than 100 mEq. per liter, thereby producing the conditions for actual bodily depletion of chloride.

(*b*) *Time of Maximum Effect.* After an injection of a mercurial diuretic, the first urine voided contained approximately the maximum concentration of chloride reached during the following 24 hours, and this level was maintained (fig. 4). This increase was seen in all of four experiments. A change in urinary chlorides was usually demonstrable in the 48 hour specimen, and sometimes in the 72 hour specimen.

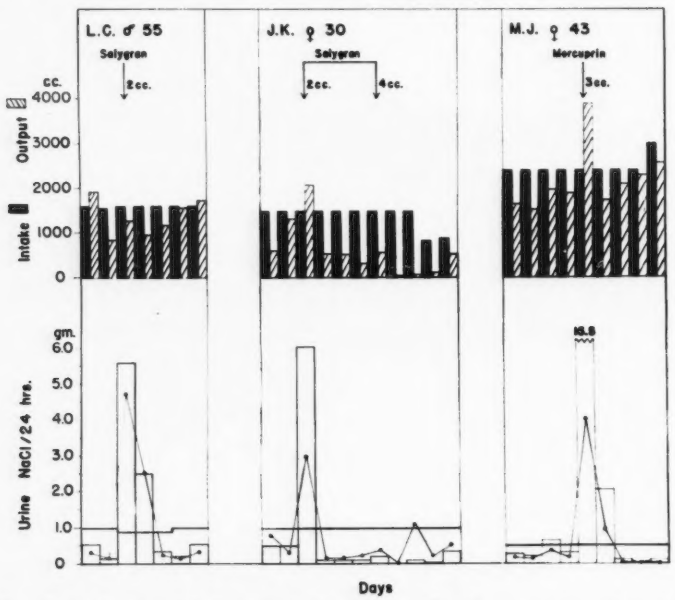


FIG. 3. The effect of mercurial diuretics in 3 subjects. The dose used was minimal in its effect on the excretion of water. The straight horizontal line at the bottom of the chart indicates the level of intake of sodium chloride in the diet. The injection of Salyrgan in patient L. C. resulted in a negligible increase in urinary volume but a marked increase in both the amount of urinary chloride (shown by blocks) and the concentration (shown by dots). In patient J. K. Salyrgan produced a similar response on the first injection with somewhat more change in the urinary excretion of water. A second injection four days later of twice the amount failed to produce the response either in the excretion of water or chlorides. At this time plasma chlorides were at low levels (83 mEq. per liter). The usual response is shown in patient M. J. whose urinary excretion of water approximately doubled while that of chloride increased over forty fold.

(*c*) *Effect on Extracellular Electrolytes.* The injection of a mercurial diuretic was followed by a slight although definite fall in plasma

chlorides in 4 patients whose diets were restricted as to salt (table 3). This effect was also seen after one of two injections in a case in which edema fluid was being collected by Southey tubes; the chloride content of the edema fluid decreased markedly, although drainage was unaffected. Frequently seen was a depression of the chloride content of the urine three to four days after the injection, which sometimes reached negligible levels. No

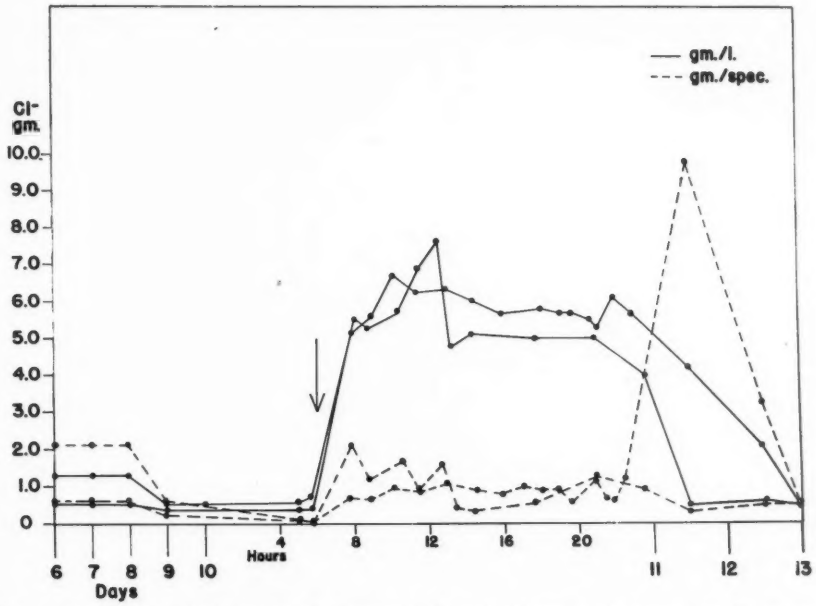


FIG. 4. The effect of the injection of a mercurial diuretic (shown at the arrow) on the content of chlorides in single urine specimens (dotted line) and on the concentration per specimen (solid lines) of 2 patients suffering from severe congestive circulatory failure. The high chloride content of one patient 24 hours after injection reflects continuation of chloruresis, and the fact that single specimens were not measured separately.

TABLE 3.—*Effect of Mercurial Diuretics on Extracellular Electrolytes*

Case No.	Before		Drug and Amount	After			Change	Remarks
	Plasma	Edema		8 hrs.	24 hrs.	48 hrs.		
	Cl mEq./L.	*Cl mEq./L.*	*cc.*	*Cl mEq./L.*			*mEq./L.*	
32—J. V. ♀	97.8		3.0 (S)	95.0			−2.8	Congestive heart failure A. H. D.
45—G. S. ♂	98.2		2.0 (M)	95.6	95.6		−2.6	Normal
54—A. G. ♀	100.8		2.0 (M)		102.2		+1.4	Mod. arterial hypertension
50—V. H. ♀	101.6		2.0 (M)		100.4		−1.2	Mod. arterial hypertension
24—E. C. ♀		89.6	4.0 (S)	89.2	89.6	90.6	+1.0	R. H. D. Aur. Fib. Congestive
		83.1	4.0 (S)	79.7	77.9	76.6	−6.5	heart failure
8—B. H. ♂	97.0		4.0 (M)		95.0		−2.0	A. H. D. Congestive heart failure

Note: (S) = Salyrgan; (M) = Mercupurin.

The intake of sodium chloride in the diet was 1.0 Gm. in all cases but 54 (A. G.), whose diet was unrestricted as to salt.

greater chloride diuresis was caused by mercurial diuretics when plasma chlorides were artifically elevated from low normal levels in two experiments, but when they were markedly depressed no measurable chloride appeared in the urine.

(*d*) *Comparison of Different Diuretics.* Salyrgan, Salyrgan-theophylline, and Mercupurin acted similarly and to the same extent on the renal excretion of chloride (fig. 2). When larger doses were given, immediate effects were different. Reactions were observed when compounds containing theophylline were used intravenously. Patients complained of dyspnea, orthopnea, and palpitation; tachycardia was sometimes observed. When venous pressure was measured, a decided fall occurred, immediately after Salyrgan-theophylline and later after Mercupurin, a change not seen with Salyrgan (fig. 5). Two patients died suddenly during this period. Both suffered from severe congestive failure, with ascites, hepatic insufficiency and many irregular cardiac beats from ectopic ventricular foci. It is believed that these reactions were caused by the immediate circulatory and cardiac effects of theophylline, liberated rapidly from Salyrgan-theophylline and more slowly from Mercupurin. The amount of theophylline contained in the dose used (3.0 to 6.0 cc.) was sufficient to act upon the circulation (106 to 212 mg. for Mercupurin and 150 to 300 for Salyrgan-theophylline).

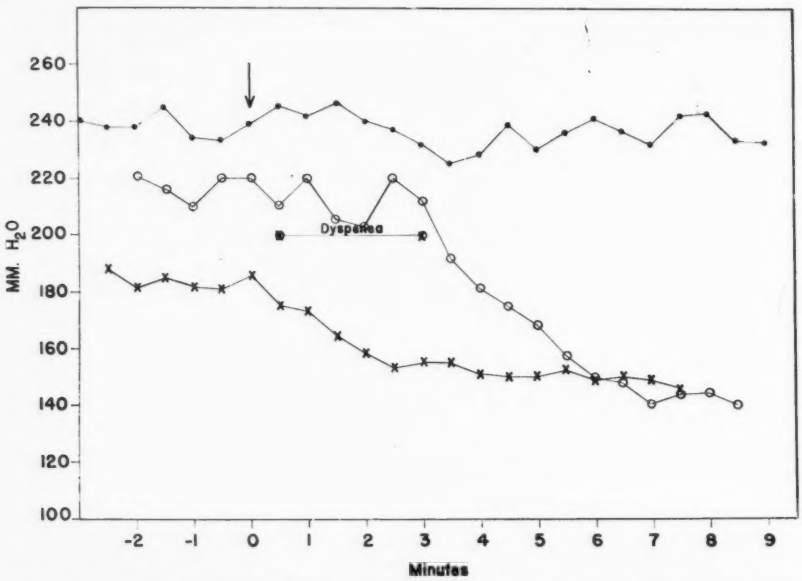


FIG. 5. The effect of the injection of various mercurial diuretics on venous pressure. The dots indicate that salyrgan was used, the open circles Mercupurin and the crosses Salyrgan-theophylline. The injection was made at the arrow and the dose was the same in all 3 instances (4 cc.). All injections were made in the same patient, B. H. Dyspnea, palpitation and tachycardia occurred after the injection of the compounds containing theophylline.

(*e*) *Augmentation of the Action of Mercurial Diuretics by Ammonium Nitrate.* In 5 subjects the actions of mercurial diuretics on chloride excretion was compared before and during the administration of ammonium nitrate (table 4). Although difficult to evaluate, as the state of circulatory failure varied from time to time, a marked increase in urinary chlorides was noted in 3 of the patients, a slight increase in one, and a slight depression in one when ammonium nitrate was given. Urine volume changed proportionately to the change in chlorides. Ammonium nitrate alone acted as a mild chloride diuretic in 2 of the 5 cases.

(*f*) *Effect of Mercurial Diuretics on Pulse Rate.* Control of edema by injections of Salyrgan did not decrease the pulse deficit in one patient exhibiting rapid auricular fibrillation, but did was no demonstrable change. Of eighteen unequivocal experiments in patients with normal rhythm, the rate was lowered to a similar degree in 8.

TABLE 4.—*Effect of Ammonium Nitrate on the Excretion of Chloride Induced by Mercurial Diuretics*

Case No.	Control			Ammonium Nitrate			Dose (cc.)
	Output Cl (NaCl)		Urine Vol. 2 days	Output Cl (NaCl)		Urine Vol. 2 days	
	Gm./2 days	Gm./L.		Gm./2 days	Gm./L.		
22—C. M. ♂	3.56	3.70	961	22.03	5.79	3805	2.0 (M)
18—E. K. ♂	29.42	5.66	5186	23.82	5.21	4572	5.0 (M)
19—M. J. ♀	6.87	2.39	2884	25.60	4.29	5969	5.0 (M)
53—F. R. ♀	5.64	4.34	1300	18.57	6.02	3090	2.0 (M)
25—W. D. ♂	12.88	4.42	2425	13.00	4.46	2917	4.0 (M)

(M) = Mercupurin—The same dose was given in each instance. Ammonium nitrate was administered in doses of 8.0 Gm. per day for 3 to 4 days prior to the injection of Mercupurin, and 3 days subsequently.

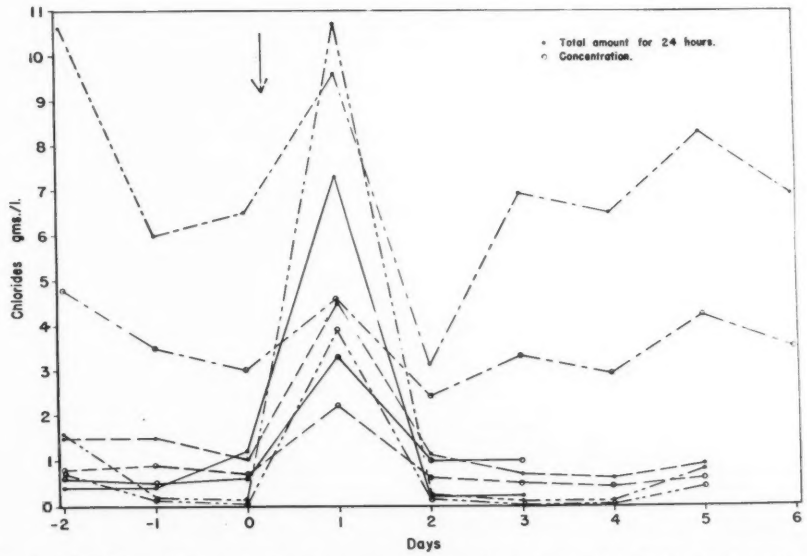


FIG. 6. The effect of the injection of a mercurial diuretic in 4 normal individuals. Injection was made at the arrow. The concentration and total urinary excretion is shown. The two upper curves indicate the effect on a subject ingesting 8 Gm. of sodium chloride per day. The change is not striking. The remainder of the subjects were on a diet containing 1 to 2.0 Gm. sodium chloride per day. Note that there is an increase in both the amount and concentration in all cases.

lower the rate. Digitalis later controlled the pulse deficit without affecting edema. In 5 others with this arrhythmia, the average ventricular rate was below 100 beats per minute, and was decreased 10 or more beats per minute after mercurial diuretics were given; in 10 there

(*g*) *Location of Mercury in Various Organs.* Tissues were analyzed for mercury in the 2 patients who died shortly after an injection of Mercupurin containing a total of 156 mg. of the metal. In one, mercury was found distributed principally in the liver (2.0 mg. per

100 Gm.), heart muscle (1.0 mg. per 100 Gm.), and lungs (0.8 mg. per 100 Gm.). The kidneys contained only a trace (0.05 mg. per 100 Gm.), and none was found in fat. A similar distribution measured qualitatively was found in the other case. Two patients received many injections of various mercurial diuretics in large doses (4 to 6 cc.), usually at weekly intervals over many years. No mercury was found in their kidneys at postmortem examination, nor were there pathologic lesions suggestive of mercury poisoning.

(*h*) *Effect on Normal Subjects.* Mercurial diuretics were given to 6 normal or hypertensive subjects with normal cardiac and renal function. When the dietary intake of salt was high, little significant effect could be observed on the daily variations of chloride excretion, although the amount always rose slightly on the day the drug was given. When salt was restricted, however, these agents produced an effect identical to that seen in cardiac patients, i.e., an increase in the urinary concentration and excretion of chlorides (fig. 6). Water, however, was much less affected. The masking of the action of these drugs in subjects taking 5 to 8 Gm. of salt per day is probably due to the inability of their kidneys to increase the concentration of urinary chlorides to a level much above that already present.

Discussion

An examination of the action on urinary chlorides and water of the three diuretic agents employed in this study revealed variations requiring explanation.* Since the patients studied had retained water and salt (edema fluid) in their bodies, the effectiveness of therapy could be estimated both by measurements of the volume of water and salt excreted by the kidneys and by alterations in body weight (fig. 7). It has not yet been established whether retention of sodium, of chloride, or of both is primarily involved in the causation of increased extracellular fluid, although sodium (balanced by chloride) is believed to be the principal ion. Studies of chloride balances, however, probably reflect fairly adequately alterations in the amount of extracellular fluid in the absence of alkalosis or acidosis, especially when edema is present and diuresis occurs. The assumption is made that the method of evaluation of diuretic agents used in this study has enough validity for conclusions to be drawn, in spite of disregard of chloride losses by other (feces, sweat) than urinary routes. Urinary volume alone has been found to be an unreliable index of changes in extracellular fluids.[2]

In the light of these experiments and of existing knowledge concerning the action of diuretic agents, it becomes necessary to examine the possible effects of these drugs upon the several organs of the body whose derangement may lead to the formation of edema, that is, the heart, kidneys,[6] adrenal glands, and organs leading to the release of antidiuretic substances. Each drug must therefore be scrutinized from the viewpoint of its primary action. Newer knowledge may bring to light effects upon other systems more remotely concerned.

1. *Digitalis.* The primary action of digitalis is upon cardiac muscle, lengthened fibers being made shorter and the force of their contractions probably being increased. Direct action on the kidneys has not been conclusively demonstrated. The glycosides of digitalis, however, resemble chemically the steroid hormones and may be involved in certain enzymatic processes. Effects of digitalis upon electrolyte disturbances can probably be ascribed to the general improvement in the circulation which follows their use. In the present controlled experiments, the results were disappointing. A therapeutic response, however, may be accompanied by an increase in urinary volume (2 cases), an increase in the output of chlorides (2 cases), or both (4 cases). No specific function of the kidneys therefore was affected; presumably the

* It is not within the province of this report to discuss the voluminous literature concerning the mechanism of action of these three diuretic agents. The reader is referred to Pitts and Sartorius[4] for a concise summary of the evidence, which is, in some cases, far from clear. The results of these experiments would agree, in the main, with the known facts concerning the action of digitalis and mercurial drugs, but disagree in part with the explanation offered as to the action of xanthines. It is possible that the action of diuretic agents on normal kidneys of animals may be somewhat different from that on the kidneys of patients suffering from congestive failure.

function of the myocardium improved and thereby provided a more adequate renal circulation.

The relative ineffectiveness of digitalis can be explained in part by the type of patient studied. "Right sided" failure was present in all cases, and was predominant in that pulmonary congestion or edema was not an important symptom. The amount of extracellular fluid present was usually large, 6 patients exhibiting ascites. Myocardial damage of an irreversible nature was found in 11; 3 exhibited signs of insufficiency of the tricuspid valve as well as of rheumatic involvement of the mitral valve. Although these cases represented congestive circulatory failure of cardiac origin, their signs and symptoms pointed to a permanent deficiency predominantly in the output of the right ventricle. Digitalis in such circumstances might be expected to be relatively ineffective. These studies suggest that its value in severe chronic congestive circulatory failure with normal rhythm may be more apparent than real; the usual practice of combining this drug with measures such as low salt diets and bed rest, may mask a lack of response which would only appear in controlled studies such as these.

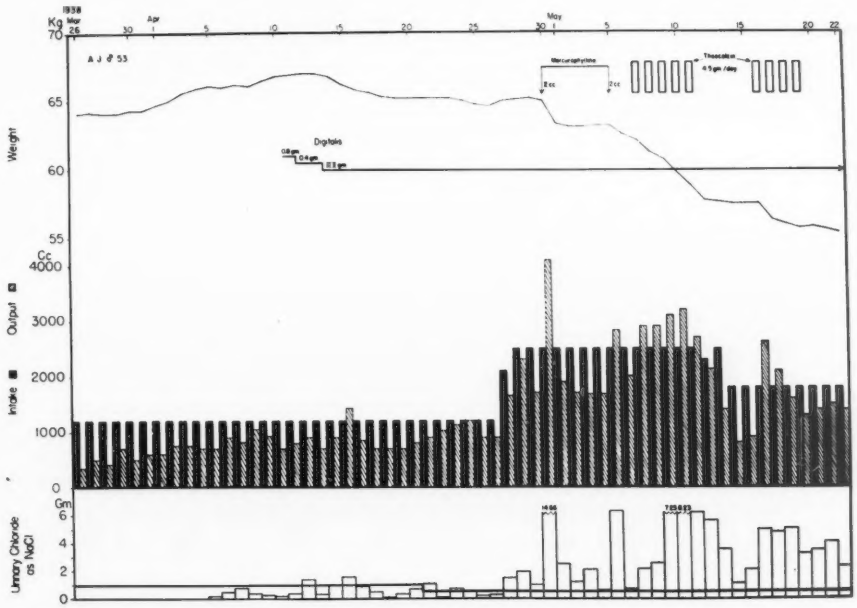


FIG. 7. Comparative actions of digitalis, mercurophylline (Mercupurin) and Theocalcin upon chloride and water excretion and body weight in the same individual, a 53 year old man with congestive failure due to rheumatic heart disease. The amount of edema was estimated at over 12 liters but there was no ascites or fluid in the pleural cavities. Decompensation had been present at intervals for several years. The horizontal line at the bottom of the chart indicates the dietary intake of sodium chloride which for a time was 1.0 Gm. per day and later 0.5 Gm. per day. Digitalization resulted in a change in the curve of body weight and a slight chloruresis and little if any effect upon the urinary output of water. Mercupurin resulted in marked chloruresis after the first injection and only a moderate response after the second. Theocalcin acted upon both water and chlorides regardless of the intake of the former and apparently caused a marked decrease in body weight. The patient was in bed during the period of study.

2. *Theocalcin.* The action of xanthine diuretics in human beings suffering from congestive circulatory failure has not been satisfactorily explained. In animals[7] and man[8] they appear to inhibit the reabsorption of salt by the renal tubules, although their renal circulatory effects (vasodilatation) may be important

Some of the experiments reported here are suggestive of a different action. The most noticeable effect of Theocalcin was on the urinary volume, which increased in every case, to a significant degree in fourteen of seventeen experiments. Urinary chlorides paralleled the increase in water excretion in eleven experiments, but their concentrations became more than 1.0 Gm. per liter in only two and more than 2.0 Gm. in only one.

The diuresis of water caused by Theocalcin, however, was not accompanied by chloruresis in six experiments, the concentration not increasing significantly. Whatever the intrarenal mechanism affected, this drug appeared to cause a consistent effect upon the renal excretion of water which sometimes, but not always, was associated with changes in the renal excretion of chlorides. Possible mechanisms involved include (a) an increase in glomerular filtrate without an effect upon tubular reabsorption of salt, (b) inhibition of the action of antidiuretic hormones, (c) improvement in the output of the heart affecting renal blood flow, and (d) intrarenal vasodilatation especially of afferent arterioles. This agent has been shown by the present experience to be one of the better diuretic drugs for the removal of edema, having an action which may be especially valuable in a certain set of circumstances, that is, when removal of excess water is more desirable than removal of excess salt.

3. *Mercurial Diuretics.* In so far as is known, mercurial diuretics act by inhibiting renal tubular reabsorption of salt, probably interfering with some enzyme system concerned with cellular transport from tubular lumen to capillary blood. It has not yet been established whether sodium or chloride ions are principally affected, although there is a little evidence in favor of the latter.[9] Conclusive experiments showing other than renal actions of these drugs have not been made. Their specific effect upon chloride excretion, reported previously,[3] is established by the present observations, which further show that they may cause concentrations in the urine greater than that in edema fluid, and therefore induce relative chloride depletion. This fact becomes important when they are employed in conjunction with low dietary intakes of salt, for the "low salt syndrome" may develop.[5]

The action of mercurial diuretics therefore differs from that of the other two drugs discussed. Although water was usually carried with salt, by grading doses it was possible to cause increased excretion of chlorides without alteration of the volume of urine; the net effect was to increase the *concentration* of urinary chlorides.* Even when these agents were relatively ineffective on the output of water (cf. cases C. M., M. J., and F. R., table 4) the concentration in the urine increased manyfold. Weight was always lost following their use except in terminal states exhibiting low electrolytes in plasma and when depletion of body salt was severe[5]; under these circumstances urinary volume and chlorides were unaffected by mercurial diuretics.

4. *Changes in Cardiac Rate.* The response of the average cardiac rate to both digitalis and the mercurial diuretics was similar. The rate in 6 of 11 patients with normal sinus rhythm was slowed by digitalis. Mercurial diuretics lowered the rate to a similar extent in 8 of 18 subjects. In patients with auricular fibrillation and a slow ventricular rate, digitalis caused further slowing one of four times; mercurial diuretics likewise caused slowing six of sixteen times. Digitalis was more effective when the rate was rapid. Theocalcin, however, caused tachycardia. If the cardiac rate is a function of the degree of congestive failure and the amount of pressure in the right auricle, it may

* The maximal concentrating ability of normal kidneys for sodium chloride is about 1.8 Gm. per 100 cc. (307.8 mEq. per liter). The highest concentration of chloride measured after mercurial diuretics were given (135.4 mEq. per liter) approached normal levels of plasma sodium, and exceeded considerably levels of plasma chloride (100 mEq. per liter). Under no experimental conditions were the kidneys of patients suffering from congestive failure able to concentrate chlorides to degrees approaching those of normal kidneys. When digitalis and xanthine diuretics were used the extremely low concentrations rose only slightly or moderately. In other words, concentrations of salt in the urine excreted following mercurial diuretics approached or exceeded the levels in edema fluid (or glomerular filtrate), while those during xanthine or digitalis diuresis were always relatively low.

be affected by the volume of circulating blood and become slower when venous return to the heart is lessened. A rapid ventricular rate with a large pulse deficit was little affected, however, by mercurial diuresis, suggesting that the intrinsic cardiac disturbance causing fibrillation was not entirely dependent upon the presence of failure.

The therapeutic implications of this study deserve comment. If the purpose of therapy is to remove excess salt, the mercurial diuretics are the drugs of choice. If excess water is to be removed, the xanthine diuretics appear to fulfill that function. If the primary cause of the failure is strictly "cardiac" and involves myocardial dilatation, digitalis may be of value. Obviously these drugs should be employed to exploit their primary actions, and not used indiscriminately to control a state of edema resulting from one or several causes.

Summary and Conclusions

1. Diuretic agents—digitalis, a xanthine (Theocalcin), and mercurials—were given to 46 patients, 40 of whom suffered from congestive circulatory failure of cardiac origin. The renal excretion of water and chlorides was measured daily, and dietary intakes of salt, water and food controlled at constant levels.

2. In 5 of 15 subjects, digitalis appeared to be effective in controlling congestive failure. In 10 of 13, Theocalcin was effective. The mercurial diuretics almost always caused an increased renal excretion of chlorides, often in large amounts, while Theocalcin appeared to act primarily upon the excretion of water.

3. Various aspects of the action of diuretic agents were discussed in the light of the probable pathogenesis of congestive circulatory failure.

Acknowledgment

The author is indebted to Miss Tilla Hefter for performing the analyses of urinary chlorides, to Dr. Palmer H. Futcher and Dr. Kendall Emerson, Jr., for those of plasma chloride and sodium, and to Miss Julia Finn for preparing the charts.

REFERENCES

[1] Schroeder, H. A.: Studies on congestive heart failure. I. The importance of restriction of salt as compared to water. Am. Heart J. **22:** 141, 1941.

[2] —: Studies on congestive circulatory failure. III. The relation of edema to urinary chlorides. Circulation **1:** 481, 1950.

[3] Futcher, P. H., and Schroeder, H. A.: Studies on congestive heart failure. II. Impaired renal excretion of sodium chloride. Am. J. M. Sc. **204:** 52, 1942.

[4] Pitts, R. F., and Sartorius, O. W.: Mechanism of action and therapeutic use of diuretics. J. Pharmacol. & Exper. Therap. **98:** 161, 1950.

[5] Schroeder, H. A.: Renal failure associated with low extracellular sodium chloride: The "low salt syndrome." J. A. M. A. **141:** 117, 1949.

[6] Merrill, A. J.: Edema and decreased renal blood flow in patients with chronic congestive heart failure: Evidence of "forward failure" as the primary cause of edema. J. Clin. Investigation **25:** 389, 1946.

[7] Walker, A. M., Schmidt, C. F., Elsom, K. A., and Johnston, C. G.: Renal blood flow of unanesthetized rabbits and dogs in diuresis and antidiuresis. Am. J. Physiol. **118:** 95, 1937.

[8] Davis, J. O., and Shock, N. W.: The effect of theophylline ethylene diamine on renal function in control subjects and in patients with congestive heart failure. J. Clin. Investigation **28:** 1459, 1949.

[9] Farnsworth, E. B., and Krakusin, J. S.: Electrolyte partition in patients with edema of various origins. J. Lab. & Clin. Med. **33:** 1534, 1948.

The Fixation of Radioactive Digitoxin by Isolated Hearts

By A. Sjoerdsma, Ph.D., M.D., and C. S. Fischer, M.D.

The nature of digitoxin fixation in isolated hearts was studied with C^{14} labelled digitoxin. Fixation was greatest in the early stages of perfusion. The amount of drug fixed was measured directly. A considerable percentage of the digitoxin in cardiac muscle was changed to other substances. These substances are more firmly bound to the heart than digitoxin.

THE preparation of pure, randomly labelled, radioactive digitoxin in this laboratory[1] has made available a sensitive tracer technic particularly suited to studying the fate of digitoxin in isolated organs and intact animals. In contrast to other methods of assay, this new technic enables one to follow the unchanged drug as well as metabolic products formed after interaction with body tissues. The extreme sensitivity permits experiments with digitoxin in amounts which are not lethal to the intact animal. Furthermore, in perfusion experiments on isolated hearts, the concentration of labeled digitoxin is low.

The work of previous investigators has not settled the problem of digitoxin fixation in cardiac muscle. Conclusions drawn were only by inference since the results depended on biologic assay experiments. With the radioactive technic, *direct* measurement of digitoxin uptake can be carried out on homogenates of hearts previously perfused with radioactive solutions. The percentage of conversion to other substances can also be determined by this direct method. Furthermore, studying the radioactivity of the perfusion fluids of isolated heart preparations enables one to judge the rapidity of drug fixation by heart muscle. With these possibilities in mind we studied the fixation of radioactive digitoxin by isolated hearts of rats, guinea pigs, rabbits and cats.

From the Department of Pharmacology, The University of Chicago, Chicago, Ill.

This work was carried out under a contract between the Atomic Energy Commission and the University of Chicago. It was aided in part by grants from the Life Insurance Medical Research Fund and the Dr. Wallace C. and Clara A. Abbott Memorial Fund.

Methods

A small amount of radioactive digitoxin with a specific activity of 350 counts per minute per μg. was supplied in a stock solution containing 50 μg. of drug per cc. of 95 per cent ethanol. For perfusion, digitoxin was diluted with Ringer-Locke solution to a concentration of 0.2 μg. per cc. The hearts were perfused by the Langendorff[2] technic through a cannula inserted into the aorta. A two-way stopcock above the cannula permitted rapid change from control to radioactive solutions.

After stabilization of the rate and rhythm, the hearts were perfused with radioactive digitoxin and six successive 25 cc. samples of perfusate were collected. Control specimens were drawn from the side arm of the cannula. Immediately following perfusion, a 10 per cent water homogenate of the hearts was made and 1 cc. volumes of homogenate and perfusion fluids were dried on flat copper discs in an area of 10 sq. cm. Measurements of the total radioactivity were carried out on these preparations.

To determine the percentage of total radioactivity due to unchanged digitoxin, extractions were done on 5 cc. aliquots of control and perfusate solutions, and on 2 to 10 cc. aliquots of heart homogenate. Each sample was shaken for 15 minutes in a glass-stoppered centrifuge tube containing 1 mg. of nonradioactive digitoxin in 20 cc. of reagent grade chloroform. After centrifuging for 10 minutes, the water phase was discarded and the chloroform fraction filtered. The residue was re-extracted twice with chloroform, filtered, and the filtrate added to the original. The total was then evaporated to dryness to remove excess water. The dried material was redissolved in 30 cc. of chloroform

and adsorbed on a 5 by 50 mm. adsorption column using alumina, which had previously been washed successively with distilled water, 95 per cent ethanol and chloroform. Thirty cc. of 1 per cent ethanol in chloroform was added to the column, followed by 50 cc. of 10 per cent ethanol in chloroform. This latter fraction was collected, dried, redissolved in 10 per cent ethanol in chloroform, transferred to a 5 cc. beaker with a Pasteur pipet, and again evaporated to dryness. Finally, the extract was suspended in 1.2 cc. of 20 per cent ethanol in water and plated on a copper cup in a circular depression 10 sq. cm. in area. By this method of extraction, recovery of radioactive digitoxin from Ringer-Locke solution, perfusate and water was 85 to 100 per cent but only 50 to 70 per cent from 10 per cent heart homogenate.

Triplicate samples were counted on copper cups and discs in the internal Geiger counter as described by Kelsey,[3] with a difference between samples of less than 10 counts per minute. This represents a maximum error of 10 per cent, the more active samples affording measurements of greater accuracy. A self-absorption correction factor was used with heart homogenate whereas self-absorption by residues from Ringer-Locke and perfusion solutions was negligible.

Results

Digitoxin in the low concentration used produced little physiologic effect. A slight depression of contractile force was noted in most cases, probably due to the alcohol in the solution. Notwithstanding the small amounts of the drug, the radioactivity was sufficient to enable accurate analysis.

By comparing the radioactivity of the solutions before and after perfusion, it was possible to obtain a measure of the rapidity of digitoxin fixation. Such data, representing an average of

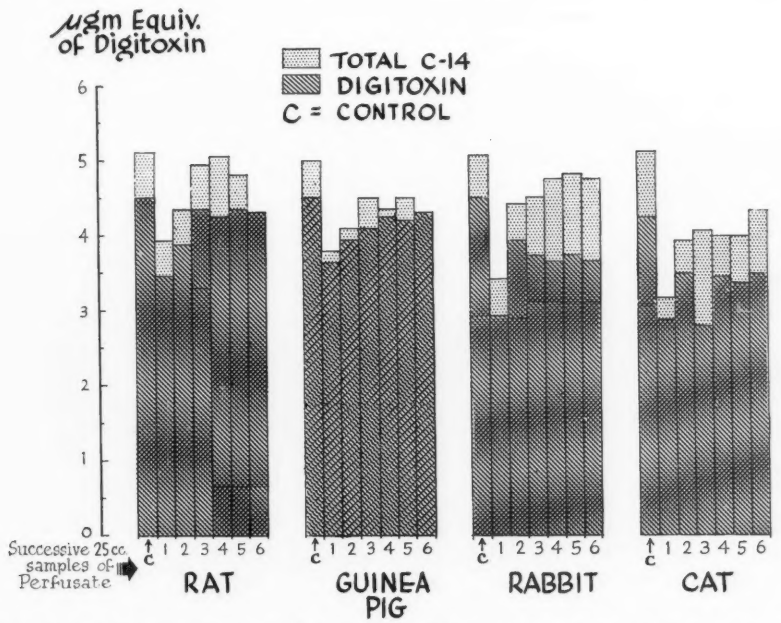


FIG. 1. *Rate of Digitoxin Fixation as Measured by Radioactivity of the Perfusing Fluid before and after Passage through Isolated Hearts.* Control is the radioactive Ringer-Locke solution perfused and the perfusates are collections of the fluid post-heart. The bars represent the total C^{14} (as digitoxin equivalent) in 25 cc. samples and the cross-hatched portion refers to the C^{14} which on extraction of the fluids is accounted for by unchanged digitoxin.

three to six experiments on each species, is given in figure 1. Fifteen to 20 minutes was required for rat and guinea pig perfusions, and 5 to 10 minutes for rabbits and cats. The bars depict the total radioactivity (expressed as μg. equivalents of digitoxin) of control digitoxin and perfusate fluids, while the cross-hatched portion refers to recoverable digitoxin. Blank experiments demonstrated small but variable losses of radioactivity in the apparatus. Hence, the values obtained do not lend themselves to an accurate calculation of total drug fixation but are given rather to show the general curves of uptake. Graphs of the four species are similar

TABLE 1.—*Average Uptakes of Radioactive Digitoxin by Isolated Hearts*

(Perfused with 150 cc. of Ringer-Locke solution containing 0.2 μg. of radioactive digitoxin per cc.)

Animal	Number of Experiments	Total C^{14} Uptake (μg./Gm.)	Percentage Recovered as Digitoxin	Total Digitoxin per Heart† (μg.)
Rat	4	1.49 (1.29–1.61)*	59 (52–74)*	1.74 (1.57–1.93)*
Guinea pig	4	1.76 (1.47–2.16)	68 (63–70)	2.63 (2.58–2.74)
Rabbit	5	0.65 (0.38–0.98)	70 (53–85)	3.66 (2.59–6.43)
Cat	5	0.83 (0.72–1.17)	62 (48–71)	8.89 (6.55–11.52)

* Range of values is indicated in parenthesis.

† Average heart weights were: rat, 1.2 Gm.; guinea pig, 1.5 Gm.; rabbit, 6.0 Gm.; cat, 10.2 Gm.

in configuration; there was a rapid initial uptake of carbon^{14} followed by a slow but persistent fixation through the course of the perfusion. The amount of digitoxin in the perfusates tended to parallel the total radioactivity, although toward the end of the perfusions the percentage of digitoxin in the perfusates was less for rabbits and cats than for rats and guinea pigs.

In table 1 are shown the uptakes of digitoxin as derived from direct measurement of heart homogenate activity. The total carbon^{14} fixed by rat and guinea pig hearts was remarkably constant and averaged an equivalent of 1.49 and 1.76 μg. of digitoxin per Gm. of heart, respectively. Fifty-nine and 68 per cent of the radioactivity was present as unchanged digitoxin. The fixation by rabbit and cat hearts was variable but the averages were .65 and .83 μg. per Gm. of heart, with corresponding percentages of 70 and 62 as digitoxin. The total fixation of digitoxin varied directly with the heart weight, so that the large cat hearts exhibited the greatest fixation.

The mode of digitoxin fixation to heart muscle is unknown. To learn whether or not digitoxin is reversibly bound, hearts of the same four species were perfused as before with 150 cc. of radioactive digitoxin solution, after which the perfusion was continued with 50, 200, 450 or 1000 cc. of nonradioactive Ringer-Locke. The hearts were then homogenized and total and digitoxin-recoverable carbon^{14} measured. The results as given in table 2 again indicate that rat and guinea pig uptakes are less variable than those of rabbits and cats. The rabbit hearts washed with 50 and 450 cc. and the cat

TABLE 2.—*Effect of Washing on the Digitoxin Content of Isolated Hearts*

Animal	Volume of Wash	Total C^{14}*	Extractable Digitoxin*	Digitoxin as % of Total C^{14}
Rat	0	1.49	0.89	59
	50	0.48	0.25	51
	200	†	†	†
	450	†	†	†
	1000	†	†	†
Guinea Pig	0	1.76	1.19	68
	50	1.35	0.69	54
	200	0.51	0.26	52
	450	0.39	0.09	23
	1000	0.34	0.02	9
Rabbit	0	0.65	0.42	70
	50	1.32‡	0.36	27
	200	0.30	0.09	29
	450	1.39‡	0.06	4
Cat	0	0.83	0.54	62
	50	1.69‡	0.41	24
	200	0.60	0.23	39
	450	0.25	0.12	48

* Values expressed as digitoxin equivalent (μg./Gm. of heart)

† Value statistically invalid (less than .015 μg.)

‡ No explanation can be offered for these high values except individual variation from the mean as described in the text.

heart washed with 50 cc. contained more activity than the average for the species without washing. Nevertheless, the carbon^{14} present as digitoxin exhibited a progressive decline in the animals studied. It was particularly easy to wash digitoxin from rat hearts while the other hearts retained significant activity even after 450 to 1000 cc. of wash. Another phenomenon observed was the tendency toward a progressive diminution of the digitoxin to total carbon^{14} ratio as the washing proceeded from 0 to 1000 cc.

Discussion

In the isolated hearts of the four species studied, the uptake of radioactive digitoxin was relatively greater in the early stages of the perfusion. This is in agreement with the experiments of Straub[4] and others on the Starling cat preparation and the excised frog heart. He states that the frog heart may absorb a fatal dose of digitoxin from the solution in one minute while the systolic arrest appears only after 10 minutes. Straub believed that the receptive capacity of heart muscle might be exhausted after one passage of digitoxin through it, providing the concentration is sufficiently high. Our results with a low concentration of radioactive digitoxin can be interpreted in terms of these two hypotheses. Following the initial rapid fixation, absorption of the drug from the solution continued because the receptive capacity of the heart had not yet been exhausted. The absence of typical physiologic effect noted in our experiments is undoubtedly due in part to the small amount of digitoxin in solution; however, the delay in digitoxin action observed by Straub may also be a factor. Recent work by Friedman and Bine[5, 6] with the embryonic duck heart tends to contradict earlier experiments. Using lanatoside C and digitoxin they demonstrated that with increasing concentrations of the drugs there was a progressive decrease in the time taken for occurrence of digitalis effect. The same authors suggest that previous data may be attendant on penetration of the glycosides into cells of the adult heart.

Measuring the fixation of radioactive digitoxin by isolated hearts gave no clue as to the reason for differences in species sensitivity. The only correlation made was that hearts tended to remove digitoxin from solution in direct relation to their mass. We have no explanation for variable uptakes of rabbit and cat heart.

The reversibility of digitoxin fixation has been a subject of great controversy for years. The studies of Issekutz,[7] Straub[4] and others on the frog heart indicated that the digitalis effect persists despite prolonged washing, and that small doses lower the threshold requirement even after a long intervening wash period. The implication was that digitoxin is irreversibly bound to heart muscle. Hatcher's work[4] with intact cats led to the conclusion that digitoxin is bound to heart muscle for several weeks. The greater susceptibility of Starling cat preparations from animals pretreated with digitoxin gave further continuity to these ideas.[4] Other authors have emphatically declared that the combination of digitoxin with heart muscle is not irreversible.[8] Kingisepp[8] performed washout experiments with digitoxin using isolated frog ventricles and showed that the action of digitoxin can be completely reversed by thorough washing. With several other glycosides the effects were more easily reversible. Paff and Johnson[9] have demonstrated the same phenomenon on chick hearts. In all these studies the important criticism is that the results depended entirely on the observation of biologic effects and in no case could it be definitely proved that digitoxin was actually washed out. Hence, the dichotomy of storage in the heart versus persistence of effect was insoluble. The radioactive technic surmounts these difficulties. Our studies on the reversibility of fixation demonstrates that the amount of both total radioactivity and digitoxin can be markedly lowered by washing, but that within the limits of the experiments some digitoxin always remains, except with the rat heart. With this animal, the total radioactivity and digitoxin are readily and completely washed out. It is of interest that the rat heart is also most resistant to digitoxin. In addition it was found that digitoxin is less strongly bound than its metabolites, the latter term referring to the radioactivity not accounted for by extractable digitoxin. It is possible that this fraction consists in part of digitoxin bound in the tissues, to the tissue protein

for example. The nature of these metabolic products awaits further investigation. Experiments now in progress show that the radioactivity not ascribable to unchanged digitoxin appears in the initial water phase of the extraction and in the 1 per cent eluate, which paper chromatographic analyses indicate consists primarily of digitoxigenin.

Summary

Isolated hearts of four mammalian species were perfused by the Langendorff technic with 150 cc. of Ringer-Locke solution containing 0.2 μg. of radioactive digitoxin per cc. The most rapid uptakes of digitoxin from the perfusing fluid occurred during the early stages of perfusion. There was a relatively constant fixation of radioactive digitoxin by rat and guinea pig hearts, whereas the uptake by rabbit and cat hearts varied considerably. About 50 to 70 per cent of the total radioactivity could be extracted from the hearts as digitoxin. Experiments on the reversibility of digitoxin fixation clearly demonstrated that a considerable percentage of the digitoxin fixed in heart muscle is changed to other substances, the nature of which is unknown. The concentration of these metabolites and unchanged digitoxin in the heart can be lowered appreciably by washing, but with the exception of the rat heart, a small quantity always remains. Digitoxin is bound less firmly to heart muscle than its metabolites.

References

[1] Geiling, E. M. K., Kelsey, F. E., McIntosh, B. J., and Ganz, A.: Biosynthesis of radioactive drugs using carbon 14. Science **108:** 558, 1948.

[2] Sollmann, T. H., and Hanzlik, P. J.: Fundamentals of Experimental Pharmacology. San Francisco, J. W. Stacy, 1939.

[3] Kelsey, F. E.: An internal Geiger counter for the assay of low specific activity samples of carbon 14 and other weak beta emitters in biological samples. Science **109:** 566, 1949.

[4] Straub, W.: Lane Lecture: Cardiac Glycosides. Stanford, Calif., Stanford University Press, 1931.

[5] Friedman, M., and Bine, R.: The delay in action of digitalis glycoside (lanatoside C). Proc. Soc. Exper. Biol. & Med. **67:** 533, 1948.

[6] Bine, R., and Friedman, M.: Quantitative detection of minute concentrations of digitoxin. Proc. Soc. Exper. Biol. & Med. **69:** 487, 1948.

[7] Issekutz, V.: Absorption and storage of digitalis substances in the heart. Arch. Path. Pharmacol. **78:** 155, 1915.

[8] Kingisepp, G.: The wash-out of cardiac glucosides from the frog's ventricle. J. Pharmacol. & Exper. Therap. **55:** 377, 1935.

[9] Paff, G. H., and Johnson, B. B.: Reversibility of digitalis action. Proc. Soc. Exper. Biol. & Med. **44:** 155, 1940.

The Deposition of Digitoxin in the Tissues of the Rat After Parenteral Injection

By René Bine, Jr., M.D., Meyer Friedman, M.D., Sanford O. Byers, Ph.D., and Catharine Bland, A.B.

Quantitative studies regarding the fate of parenterally injected digitoxin in the rat were made by assaying extracts of rat tissue by the embryonic duck heart method. The concentration of digitoxin found in heart muscle was similar to that found in lung and kidney and greater than the concentration in skeletal muscle. The greatest amount was found in the liver and suggested the importance of this organ in the excretion or destruction of digitoxin.

EXACT quantitative studies concerning the fate of any digitalis glycoside in the mammalian animal after its parenteral injection have not been reported heretofore. This has been because of the difficulty of determining the necessarily minute quantities of the drug possibly present in tissues. Recently however, by means of assaying tissue extracts with the embryonic duck heart method, we have been able to detect as little as 0.05 μg. of digitoxin in a single Gm. of tissue. This has made it possible to study quantitatively the distribution of parenterally administered digitoxin in the various organs of the rat. The preliminary results of the study are given below.

Method

Three groups of rats were injected intravenously with 1 μg. of digitoxin per Gm. of body weight. The first group (7 rats) was killed immediately, the second group (6 rats) at 60 minutes, and the third group (6 rats) at 180 minutes after injection. The heart, liver, lung, kidney, muscle, spleen and brain were removed, and a 1 Gm. sample of each tissue was submitted to a standardized method of chemical extraction.

Briefly, this method of extraction consisted of (a) grinding of the tissue sample (1 Gm.) in a mortar, (b) repeated alcoholic extractions, (c) evaporation of alcoholic filtrate (40 ml.) to dryness, (d) dissolving the residue in chloroform (5 ml.), (e) partial evaporation (to 1 to 2 ml.) and refrigeration for 30 minutes, (f) decanting the solution into a 50 ml. Erlenmeyer flask, washing the residue with 2 ml. of cold chloroform, with subsequent complete evaporation, and (g) dissolving the dry residue in 10 ml. of Tyrode's solution. From this 1:10 Tyrode's solution, dilutions suitable for the duck heart embryo were made. Almost all of the assays were made in preparations containing the extracted residue of 1 Gm. of tissue in 100 ml. of Tyrode's solution. It was found in preliminary studies that this method of extraction was capable of removing completely known amounts of digitoxin which had been added to various tissues just prior to extraction. This last finding allowed us to employ our previously established standard assay values[1] for digitoxin in Tyrode's solution.

Results

Digitoxin was found in all tissues, with the exception of the brain, immediately after its parenteral injection. The immediate deposition of the drug was greatest in the liver (see table 1), for in this organ the digitoxin content (6.0 μg. per Gm. of tissue) was three times that found in the heart (2.0 μg. per Gm. of tissue). The renal and pulmonary contents of digitoxin were identical (2.7 μg. per Gm. of tissue) immediately after injection. Comparatively little was found in skeletal muscle (0.25 μg. per Gm. of tissue) or in the spleen (0.45 μg. per Gm. of tissue).

Sixty minutes after injection, as table 1 indicates, all of the above tissues, except skeletal muscle, were found to contain less digitoxin. The digitoxin content of skeletal muscle was essentially unchanged. The brain still did not contain a detectable amount of digitoxin. The liver continued to contain far more digitoxin

From the Mount Zion Hospital, Harold Brunn Institute for Cardiovascular Research, San Francisco, Calif.

Aided by grants from The Life Insurance Medical Research Fund, Eli Lilly and Company, and the United States Public Health Service.

TABLE 1.—*The Deposition of Digitoxin in Tissues of Rat after Its Parenteral Administration*

Rat	Amount of Digitoxin (μg./Gm. of Organ Weight)						
	Kidney	Liver	Heart	Lung	Muscle	Spleen	Brain
*A. Immediately after Injection**							
242	2.5	7.5	2.5	1.0	0.25	—	N.D.†
343	2.5	5.0	2.5	2.5	0.25	0.5	N.D.
355	2.5	5.0	1.0	2.5	—	0.8	N.D.
367	2.5	5.0	2.5	2.5	—	—	N.D.
379	3.5	7.5	—	5.0	0.25	0.5	—
391	—	—	1.0	—	—	0.25	—
394	—	—	2.5	—	0.25	0.25	—
Average	2.7	6.0	2.0	2.7	0.25	0.45	N.D.
*B. 60 Minutes after Injection**							
262	1.0	2.5	2.5	1.0	0.25	—	N.D.
267	2.5	5.0	2.0	2.5	0.50	—	N.D.
271	2.5	5.0	1.0	2.5	0.25	—	N.D.
281	2.25	5.0	0.5	1.0	—	0.25	—
288	2.50	5.0	2.5	2.5	0.25	0.25	—
384	2.50	5.0	2.5	2.5	0.25	0.25	—
Average	2.20	4.6	1.85	2.0	0.30	0.25	N.D.
C. 180 Minutes after Injection‡							
403	0.5	2.5	0.2	0.25	0.05	0.10	0.1
410	0.5	2.5	0.5	0.40	0.50	0.10	N.D.
417	0.5	1.0	0.5	0.20	0.10	0.05	0.1
424	0.5	2.5	0.5	0.50	0.40	0.20	N.D.
431	—	2.0	0.2	0.50	0.40	0.20	—
452	1.0	2.0	0.5	0.50	—	0.10	0.1
Average	0.6	2.1	0.4	0.39	0.29	0.13	—

* Extracts diluted in 100 cc. of Tyrode's, except brain (20 cc. of Tyrode's).
† None detected.
‡ Extracts diluted in 20 cc. of Tyrode's.

(4.6 μg. per Gm. of tissue) than any other organ, including the heart and kidney.

Three hours after injection, for the first time, small amounts of digitoxin (0.1 μg. per Gm. of tissue) were detected in the brains of 3 of 5 rats assayed. However, most other organs (with the exception of skeletal muscle) contained far less digitoxin than that found either immediately or 60 minutes after injection.

DISCUSSION

The above results indicate that a great deal of parenterally administered digitoxin rapidly leaves the blood to enter the various tissues of the body. The very early, yet persistent, heavy concentration of the drug in the liver suggests the possibility that this organ may have considerable importance in the excretion or destruction of digitoxin.

It was of great interest to us that no special concentration of the glycoside occurred in heart muscle as compared with such organs as the lung and the kidney, although considerably more digitoxin was deposited in cardiac muscle than in skeletal muscle. The slowness with which the brain took up digitoxin might have been expected.

Not only was the heart found to contain less digitoxin per Gm. of weight after injection of the drug than various other viscera, but also

it did not appear to retain the drug more persistently than the other organs. Thus, three hours after injection the heart was found to contain no more of the glycoside than lung and considerably less than kidney and liver.

These experimental findings, of course, suggest that the peculiar cardiac actions of digitoxin are not due to the avidity of cardiac tissue for digitoxin, but rather to some qualitative property of the drug extraordinarily perceived and reacted to both by the healthy and ailing myocardium.

SUMMARY

A quantitative study of the fate of parenterally administered digitoxin in the rat was made by means of the embryonic duck heart method. It was found that large amounts of the drug rapidly left the blood stream to enter various organs, with the exception of the brain. This latter organ was not found to contain detectable amounts of digitoxin for at least three hours after injection. The heart did not appear either (a) to concentrate large amounts of digitoxin, or (b) to retain it any longer than the various other viscera tested.

REFERENCE

[1] BINE, RENÉ, JR., AND FRIEDMAN, MEYER: Quantitative detection of minute concentrations of digitoxin. Proc. Soc. Exper. Biol. & Med. **69:** 487, 1948.

Deviations of the RS-T Segment in Acute Subendocardial Injuries

By RAYMOND D PRUITT, M.D., HOWARD B. BURCHELL, M.D., AND HIRAM E. ESSEX, PH.D.

On isolated perfused canine hearts, studies were made of the electrocardiographic changes which attend injury to the endocardium of the free wall of the left ventricle and of changes which develop following extension of the injury onto the left ventricular aspect of the septal endocardium. In an epicardial lead, the depression of RS-T segment produced by a subendocardial injury of the free wall of the left ventricle disappeared almost completely when that same type of injury was extended onto the septum.

INHERENT in the dipole theory is a prediction of the influence of an acute subendocardial injury on the form of an electrocardiogram derived from an electrode placed on the epicardium at a point overlying the injury.[5] Depression of the RS-T segment would be anticipated in a tracing made with the usual polarity of the exploring and remote electrodes. In experiments designed to reduce to a minimum the complexities attending production of such lesions, support was obtained for the postulates of the theory.[3] In a recent report[4] of experiments on isolated canine heart preparations, attention was directed to certain consequences of widespread endocardial injury on the spread of the excitation process as reflected in alterations in form of the QRS complex. The results of these same experiments gave additional support to the concept formulated from earlier experiences[2] that in the presence of acute endocardial injuries the segmental shifts in epicardial leads are of limited extent compared with coincident deviations in leads made from endocardial points.

Among the factors responsible for this finding, one appears to merit particular emphasis. Reference is made to the existence in an injury involving the entire endocardial aspect of a ventricle, of a balancing of forces, consequent to the presence of two endocardial surfaces of opposite orientation with respect to an electrode placed on the epicardium of the ventricle. In figure 1 the potential changes invoked by the injury of septal surface *A* will cancel in large measure similar forces produced by the injury of the endocardium of the free wall *B* on an electrode *C* placed on the epicardium of that ventricle.

In the isolated perfused canine heart, an experiment can be developed appropriate to determining the significance of this consideration. A record of two such experiments follows.

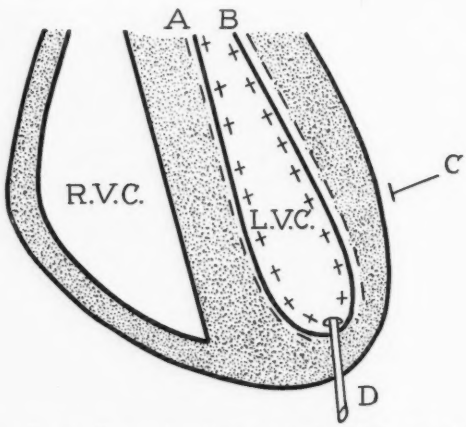


FIG. 1. For explanation, see text. R.V.C. = right ventricular cavity; L.V.C. = left ventricular cavity; C = an electrode; and D = 16 gage needle.

A foreshortened 16 gage syringe needle was placed as a drain in the most dependent portion of the apex of the left ventricular cavity (fig. 1). A wad of dry cotton was introduced into the left ventricular cavity in such a manner that the septal wall was covered. A few drops of 0.1 molar potassium chloride were then allowed to run over the endocardial aspect of the free wall of the ventricle. A continuous recording

From the Division of Medicine, Mayo Clinic, and the Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.

of electrocardiograms from the left ventricular cavity and the epicardium of the left ventricle was made. Portions of this record made before and after placing the potassium chloride on the free wall are reproduced (fig. 2). Then, without changing any of these relationships, the potassium chloride solution was permitted to run over the septal portion of the endocardial wall. The complexes recorded within a few seconds after this was done form the last set in the upper half of figure 2.

A second experiment was carried out with essentially the same results (fig. 2, lower half). The final record in that series was made after rinsing the left ventricular cavity with 0.1 normal saline solution.

Review of these records discloses that greatest depression of the RS-T segment in the lead from the left ventricular epicardium occurred in both experiments after application of the 0.1 molar potassium chloride solution to the endocardium of the left free wall. Even at this stage, segmental depression in the epicardial leads was of much lesser extent than the elevation of the segment in simultaneously recorded leads from the left ventricular cavity. When the potassium chloride solution was applied to all portions of the endocardium, the

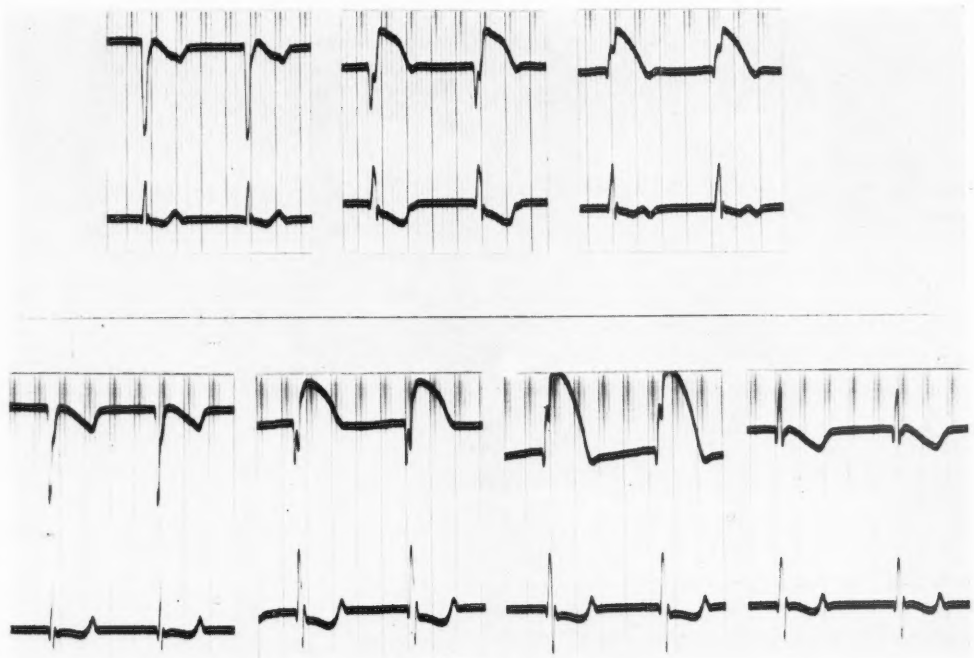


FIG. 2. *Upper half.* Tracings for experiment 1. The first row of complexes was recorded from an electrode placed in the left ventricular cavity, the lower row from an electrode on the left ventricular surface. The initial group of complexes was made after excision of the heart and institution of perfusion of the coronary circulation. The second group was recorded a few seconds after 0.1 molar potassium chloride solution was placed on the endocardium of the free wall of the left ventricle. The third group was made a few seconds after the potassium chloride solution had been placed on the endocardium of both the septum and free wall. *Lower half.* Tracings from experiment 2. The first row of complexes was recorded from an electrode placed in the left ventricular cavity, the lower row from an electrode on the left ventricular surface. The first three groups of complexes were made in same sequence as in experiment 1. The fourth and final record was made after washing the left ventricular cavity with normal saline solution.

depressed segment in the epicardial lead receded toward the isoelectric line whereas the elevated segment in the endocardial lead moved to an even higher level.

The QRS changes which occurred in these two experiments were of the same kind as those encountered in other experiments previously reported.[4]

Comment

Although an attempt was made in these experiments to isolate the consequences of a lesion affecting the endocardium of the free wall of the left ventricle from the results of a lesion involving the entire endocardial aspect of that ventricle, emphasis should rest on the point that this separation of effects almost certainly was imperfect. The first changes in each group of records represented predominantly consequences of a free wall lesion, whereas the subsequent alterations were the consequences of an unlimited lesion. In this imperfection there may lie a close kinship with the endocardial lesions of the heart subjected to severe coronary insufficiency. The electrocardiographic consequences of such lesions will depend upon their orientation with respect to an exploring electrode placed on the chest wall. In certain instances, there may occur a balancing of one effect by another so that no appreciable shift occurs in the RS-T segment. On this basis may rest the explanation for the absence of significant electrocardiographic changes in certain patients with severe coronary insufficiency when subjected to oxygen deprivation.[1] However, the more common consequences of these lesions would entail a predominance at the exploring electrode of forces of one or the other orientation. Since lesions involving the subendocardial tissues of the left free wall have the advantage of proximity to the electrode, and since such lesions produce depression of the RS-T segment in the graphic records, this type of electrocardiographic change has proved to be the usual finding in the presence of myocardial ischemia related to severe coronary insufficiency.

Summary and Conclusions

1. The electrocardiographic consequences of subendocardial injury depend upon the spatial orientation of the traumatized tissue with respect to the exploring electrode. In an epicardial lead the depression of RS-T segment produced by a subendocardial injury of the free wall of the left ventricle disappeared almost completely when that same type of injury was extended onto the septum.

2. The absence of segmental changes in the electrocardiograms of patients with coronary sclerosis subjected to hypoxia may be a consequence in some instances of this same neutralization of the effects of one area of subendocardial ischemia or injury by a similar area of opposite orientation with respect to the exploring electrode.

REFERENCES

[1] Burchell, H. B., Pruitt, R. D., and Barnes, A. R.: The stress and the electrocardiogram in the induced hypoxemia test for coronary insufficiency. Am. Heart J. **36:** 373, 1948.

[2] Pruitt, R. D., Barnes, A. R., and Essex, H. E.: Electrocardiographic changes associated with lesions in the deeper layers of the myocardium; an experimental study. Am. J. M. Sc. **210:** 100, 1945.

[3] —, and Valencia, Fernando: The immediate electrocardiographic effects of circumscribed myocardial injuries: an experimental study. Am. Heart J. **35:** 161, 1948.

[4] —, Essex, H. E., and Burchell, H. B.: Studies on the spread of excitation through the ventricular myocardium. Circulation **3:** 418, 1951.

[5] Wilson, F. N., Hill, I. G. W., and Johnston, F. D.: The interpretation of the galvanometric curves obtained when one electrode is distant from the heart and the other near or in contact with the ventricular surface. Part I. Observations on the cold-blooded heart. Am. Heart J. **10:** 163, 1934.

Oxygen Tension of Tissues by the Polarographic Method

III. The Effect of Local Heat on the Oxygen Tension of the Skin of Extremities

By Orville Horwitz, M.D., George Peirce, M.S., and Hugh Montgomery, M.D.

Simultaneous measurements of skin oxygen tension by polarography and skin temperature by thermocouple were made in patients with peripheral arterial disease and in individuals with normal extremities over a range of skin temperature of 10 to 50 C. The oxygen tension of the skin was found to increase as the skin temperature was raised to about normal body temperature in the ischemic extremity and to a significantly higher temperature in the normal extremity. Possible reasons for these changes are discussed.

OVER a period of years various authors have considered the therapeutic effects of environmental temperatures varying from 6 to 38 C. on ischemic extremities.[1-3] Those who favored the lower temperatures reasoned that lowering the metabolism was the primary consideration, while those favoring the higher temperatures were more impressed with the need of increasing the circulation by means of vasodilatation. Clinically the subjective symptom of pain and the objective sign of skin color are still the criteria by which the environmental therapeutic temperature is regulated.[4]

In polarography[5] we have at our disposal a method of measuring the amount of oxygen available at a given point in the tissue adjacent to the tip of a small platinum electrode. The range of skin temperature within which the greatest amount of oxygen is available can be found by raising skin temperature systematically while measuring skin oxygen tension polarographically. This range may not prove to be optimum therapeutically, but is worthy of consideration. Insofar as the clinically ideal skin temperature range differs from the one giving maximum oxygen availability, an adverse clinical effect of temperature upon other tissue substances than oxygen is implied. The object of this study is to discover the skin temperature range within which oxygen is most available.

From the Peripheral Vascular Section of the Robinette Foundation, Medical Clinic, Hospital of the University of Pennsylvania, Philadelphia, Pa.

This work was made possible by a grant from the U. S. Public Health Service.

Method

Ten subjects with normal extremities and 10 patients with peripheral arterial disease were studied individually. All of the patients had ischemia of the limb as judged by clinical symptoms and as measured by vasodilatation tests. (In each patient at least one pulse was absent to palpation). The leg under observation was placed in a sealed box (fig. 1) in which the air temperature could be regulated from 0 to 60 C. Four platinum electrodes (*F*) as described by Montgomery and Horwitz,[6] were inserted intradermally as shown in *F*, figure 1. Eight thermocouples for temperature measurement were distributed as follows: four (*B* and *D*) on the skin, each within 2 cm. of an inserted electrode; three (*C*) in the air in the box (*A*) within 10 cm. of one of the electrodes, and one (*E*) in the air of the room. All temperature measurements were recorded every two minutes by a Brown potentiometer connected to each thermocouple. The temperature in the box was first lowered until the skin temperature was 10 to 16 C. (fig. 2); then raised 3 degrees at a time until the skin temperature was approximately 50 C. in the case of the normal extremity, or in the case of the ischemic extremity, until intense pain required the withdrawal of the limb from the box, and an end of the study. Temperature was held constant at each new level until a constant oxygen tension was obtained.

The determination of skin temperature by this method must be subject to some error because of the influence of the air temperature as well as the skin temperature upon the thermocouple, and because surface temperature differs slightly from

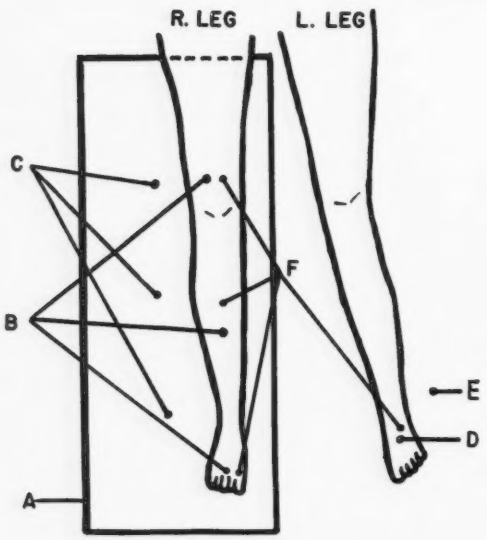


FIG. 1. Loci of insertion of electrodes and placement of thermocouples for study of right leg. *A*. Sealed box in which air temperature may be controlled. *B*. Points on right leg where thermocouples were placed for measurement of skin temperature. *C*. Points in the air inside the box where air temperature was measured. *D*. Point on left leg where skin temperature was measured by thermocouple. *E*. Thermocouple for measuring room temperature. *F*. Points of intradermal insertion of electrodes for measuring oxygen tension.

intracutaneous temperature. Such an error must necessarily decrease as the difference between skin temperature and air temperature decreases.

RESULTS

Results Common to Normal and Ischemic Extremities. As the temperature of the skin was lowered from about 35 to about 10 C., oxygen tension decreased by at least 60 per cent of the original (35 C.) value. As the skin temperature increased from about 10 C. to about 50 C., there was invariably an increase of oxygen tension of the skin by at least 1000 per cent of the value at the lower (10 C.) temperature. An increase of skin temperature above 38 C. produced the familiar reddening so ably described by Lewis.[7] In all cases in which the skin temperature was allowed to reach 46 C. the oxygen tension had started either to diminish or to level off (figs. 3 and 4). As a limb was heated or cooled, changes in oxygen tension appeared to lag behind changes in temperature. The temperature of the skin of the limb in the sealed box tended to remain closer to its original temperature than did the temperature of the air in the box (fig. 2).

Results in Normal Extremities. No normal subjects experienced pain although in most cases skin temperatures were raised to 50 C. Reversal of oxygen tension invariably occurred at 45 C.

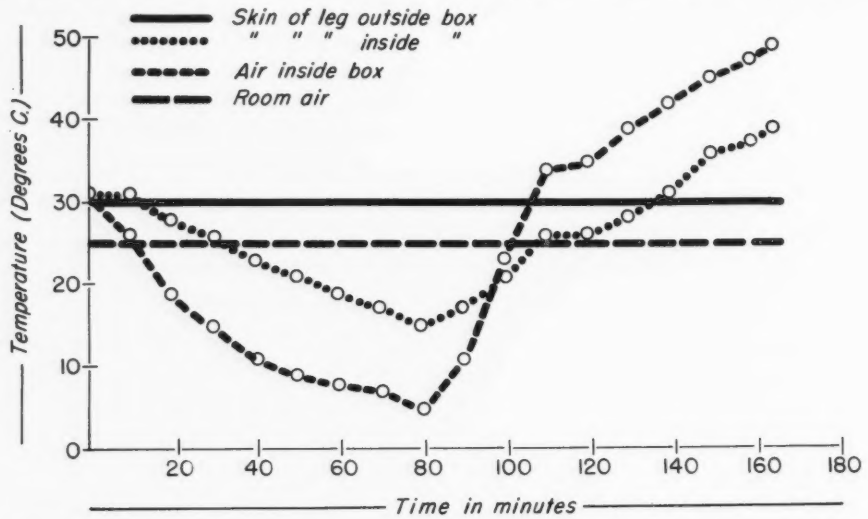


FIG. 2. Illustrating relationship between skin temperature in sealed box, skin temperature in room air and air temperature inside and outside the sealed box, in the case of subject S. A.

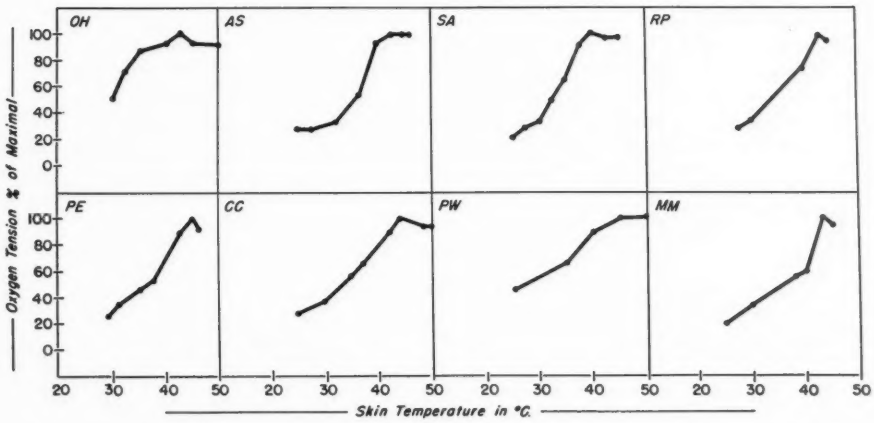


FIG. 3. Illustrating relationship between oxygen tension and temperature of the skin of normal extremities in 8 of the 10 subjects. Oxygen tension readings are expressed on a relative rather than on an absolute basis with 100 representing the maximum value obtained by any one electrode. Standard deviations of similar electrodes (in dead skin) were shown to be slightly less than 10.[6] Each determination was corrected for the physical effect of temperature upon the electrode reading.[6] Skin temperatures below 20 C. are not graphed here.

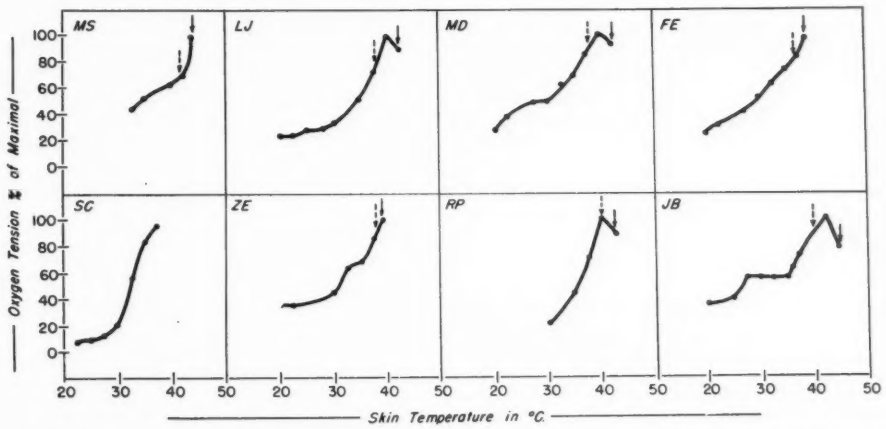


FIG. 4. Illustrating relationship between oxygen tension and temperature of the skin of ischemic extremities in 8 of the 10 patients. Oxygen tension readings are expressed on a relative rather than on an absolute basis with 100 representing the maximum value obtained by any one electrode. Standard deviations of similar electrodes (in dead skin) were shown to be slightly less than 10.[6] Each determination was corrected for the physical effect of temperature upon the electrode reading.[6] Broken arrow, pain first noted; solid arrow, pain unbearable. Skin temperatures below 25 C. are not graphed here.

or lower. For statistics of these results see table 1.

Results in Ischemic Extremities. All patients experienced local pain if the temperature of the ischemic area was raised to 39.5 C. The pain invariably became unbearable if the temperature was raised to 45 C. (fig. 4). In half of the patients oxygen tension began to decrease even though some pain was experienced first (fig. 4). However, such reversals were not noted in cases where unbearable pain necessitated cessation of the study. Neither the pain nor the

reversal of oxygen tension were necessarily present in the nonischemic areas to which heat was applied.

TABLE 1.—*Results Obtained in Normal Subjects and in Patients with Ischemic Extremities*

Effects	Number of Subjects	Average skin T. producing effects*	Stand. Dev. of T. producing effects	Average simultaneous T. of air in sealed box
		(°C.)	(°C.)	(°C.)
1. Oxygen tension reversal in normal subjects (no pain)	10	43.1	0.6	47.9
2. Oxygen tension reversal in patients with ischemic extremities	5	40.6	0.6	45.3
3. Occurrence of pain in patients with ischemic extremities	9†	38.9	0.6	44.2
4. Unbearable pain in patients with ischemic extremities	9†	42.0	0.8	48.1

* Statistically significant difference between Nos. 1 and 2. No statistically significant difference between Nos. 2 and 3.

† In the case of SC (see fig. 4) it became necessary to terminate the study for reasons other than pain.

DISCUSSION

These studies indicate that up to a certain temperature, (within one or two degrees of normal body temperature) oxygen tension increases as skin temperature increases. Tissue oxygen tension must depend upon (a) delivery of oxygen to the tissue and (b) utilization of oxygen by the tissue. From our experimental data we can infer that as temperature increases, the delivery of oxygen to the tissue increases at least with skin temperatures up to near normal body temperature. Considering the determinants of skin oxygen tension in more detail we know that:

(A) Oxygen delivery to tissue depends on the following factors:

(1) Blood flow to the tissue. Local vasodilatation, and therefore increased blood flow, occurs as a result of heat. The circulation, however, can obviously be increased to a greater extent in normal limbs, than in patients with peripheral arterial insufficiency.

(2) Dissociation of oxygen from hemoglobin, which in turn depends on: (a) The saturation of hemoglobin in arterial blood.[8, 9] In all the patients studied the hemoglobin of the arterial blood may be assumed to be 95 per cent saturated with oxygen since none of them had any demonstrable cardiorespiratory disease. (b) Temperature of the peripheral blood.[10] Increase of temperature up to at least 43 C. augments the dissociation of oxygen from hemoglobin.[10] (c) The pH of the peripheral blood.[11] A slight decrease of pH of capillary blood resulting from an increased metabolism [see (B) below] will also augment the dissociation of oxygen from hemoglobin.[11] Hence it is possible that an increase in metabolic utilization of oxygen by the tissue may be in itself a factor favoring the delivery of oxygen.[11]

(B) Utilization of oxygen by the tissue. Tissue metabolism is the main factor in decreasing the available oxygen. Gessler[12] has shown that in vitro metabolism of skin increases as temperature rises from 34 C. to 48 C. He also showed that at 48 C. the metabolism of skin begins to decrease and that at 52 C. metabolism stops. It is entirely possible, however, that increases in metabolism may also be an indirect factor in increasing oxygen tension through the medium of pH changes (above) and other mechanisms as yet unknown.

There is no reason to believe that skin oxygen is lost to environmental air since the tension of oxygen in air is greater than that in skin.[6] One cannot altogether exclude small exchanges of oxygen between skin and subcutaneous tissues. The diffusion of oxygen of air to skin is known to be small[6] in relation to the large increments in skin resulting from increments in skin temperature.

Doubtless all these variables play a part in producing the results shown in figures 3 and 4. However, no quantitation of the separate factors in intact skin is as yet available.

Because of the possible temperature error and the considerable standard deviation of the electrode itself (see Method), we do not intend to establish a specific temperature at which

oxygen tension of the skin ceases to rise in response to local heat, either in the normal or the ischemic extremity. Our data, however, do show that as the temperature of the skin increases as a result of heat applied locally more oxygen is available to skin tissue, until the temperature of the skin is raised to about body temperature in an ischemic extremity or to a significantly higher temperature in the normal extremity.

Summary

1. The oxygen tension of the skin of human extremities was measured over a range of skin temperature from 10 to 50 C.

2. Skin oxygen tension invariably became extremely low when skin temperature was lowered to 15 C.

3. In normal extremities oxygen tension of the skin increased as the skin temperature rose to several degrees above body temperature. Further increases in skin temperature reduced oxygen tension. No pain resulted.

4. In extremities with occluded arteries oxygen tension in the skin increased with rising skin temperatures up to approximately body temperature. Further increases in skin temperature caused pain that prevented continuation of the heat but did not necessarily result in decreased oxygen tension.

5. Maximal oxygen tension of the skin of an extremity made ischemic by arterial occlusive disease results from raising local temperature as high as possible without producing pain.

Acknowledgments

The authors gratefully acknowledge technical aid rendered by Miss Alice Frey.

References

[1] Bazett, H. C.: Some principles involved in treatment by heat and cold. M. Rec. **147:** 301, 1938.

[2] Barker, N. W.: Physical agents in treatment of circulatory diseases of extremities. Arch. Phys. Therapy, X-ray and Radium. **17:** 554, 1936.

[3] Large, A., and Heinbecker, P.: Refrigeration in clinical surgery. Ann. Surg. **120:** 707, 1944.

[4] Starr, I.: On the use of heat, desiccation and oxygen in the local treatment of peripheral vascular disease. Am. J. M. Sc. **187:** 498, 1934.

[5] Davies, P. W., and Brink, F., Jr.: Microelectrodes for measuring oxygen tension in animal tissues. Rev. Scient. Instruments **13:** 524, 1942.

[6] Montgomery, H., and Horwitz, O.: Oxygen tension of tissues by the polarographic method. I. Introduction: oxygen tension and blood flow of the skin of human extremities. J. Clin. Investigation. **29:** 1120, 1950.

[7] Lewis, T.: The Blood Vessels of the Human Skin and Their Responses. London, Shaw & Sons, Ltd., 1927. P. 141.

[8] Riley, R. L., Lilienthal, J. L., Jr., Proemmel, D. D., and Franke, R. E.: The relationships of oxygen, carbon dioxide, and hemoglobin in the blood of man: oxyhemoglobin dissociation under various physiological conditions. J. Clin. Investigation **25:** 139, 1946.

[9] Penneys, R.: Skin oxygen tension and arterial oxygen saturation in vivo; similarity to the oxygen dissociation curve of blood. Presented at the Physiol. Soc. of Philadelphia. Nov. 21, 1950.

[10] Brown, W. E. L., and Hill, A. V.: The oxygen dissociation curve of blood, and its thermodynamical basis. Proc. Roy. Soc. London, s. B. **94:** 297, 1923.

[11] Henderson, L. J.: Blood. A Study in General Physiology. New Haven, Yale University Press, 1928.

[12] Gessler, H.: Uber die Gewebsatmung bie der Entzündung. Arch. f. exper. Path. u. Pharmakol. **91:** 366, 1921.

Experimental Reversal of Capillary Blood Flow

By Ray Heimbecker, M.D., Vivien Thomas and Alfred Blalock, M.D.

Beck, Sciaroni and others have made clinical and experimental attempts to reverse the direction of flow of blood across the capillary bed by the anastomosis of arteries and veins. The present authors have shown by the microscopic observation of capillaries in acute experiments on cats, dogs and rabbits that a reversal of blood flow in mesenteric capillaries will occur following the connection of an artery to a vein provided the collateral arteries and veins are obstructed. Furthermore, it was shown that blood flowing in a reverse direction through capillaries loses oxygen.

A NUMBER of recent investigations in vascular surgery have been based on the hypothesis that reversal of blood flow in capillaries is possible. Experimental and clinical attempts have been made to reverse the direction of the flow across the capillary bed by the anastomosis of an artery to the venous side of the capillary bed. Several groups of investigators have anastomosed a systemic artery to the coronary sinus of the heart in attempts to cause a retrograde flow of blood through the capillaries of the myocardium.[1–4] Sciaroni[5] and Beck[6] have created an anastomosis between the carotid artery and jugular vein in an attempt to increase the blood flow to the brain. Efforts to increase the arterial blood supply to the leg were made by Johnston and associates[7] in operations in which they anastomosed the proximal end of the femoral artery to the distal end of the femoral vein.

Both the clinical and experimental studies have given results which are difficult to interpret. The present study was undertaken in an effort to determine whether reversal of the capillary blood flow is physiologically possible.

The capillary bed was observed microscopically both before and after reversal of the circulation in the living tissues of the experimental animal. In addition to the observations on the capillaries, the rate of blood flow and the oxygen consumption of the part were determined.

From the Department of Surgery of the Johns Hopkins University and Hospital, Baltimore, Md.

One of us (R. H.) was a Fellow of the National Research Council of Canada, 1949–50.

Methods

Cats were used in most of the experiments and additional studies were performed on dogs and rabbits. Anesthesia was produced by the intraperitoneal injection of Nembutal. Several regions of the body were studied in preliminary experiments but we shall report only those which were made on the mesenteric blood vessels since they yielded the most conclusive results. A short low midline incision was made and the terminal ileum was delivered, thus exposing the mesenteric blood vessels of this area. The blood vessels of the ileocecal region were followed back to the root of the mesentery and large branches of both the mesenteric artery and vein were prepared for cannulation. The femoral artery and vein were similarly prepared. The method is illustrated in figure 1.

The cannulas consisted of polyethylene tubing of suitable caliber. The largest tubing that would fit the blood vessel was used in each case, the outside diameter varying from 2 to 4 mm. The cannulas were prepared by beveling the tips with a sharp scalpel, after which they were treated with Drisilicone. Short couplers of larger tubing, which fitted snugly over the cannulas, were used to connect them together. As can be seen in the illustration in figure 1, ligatures of braided silk were placed around the bowel and its remaining mesentery at the extreme ends of the segment of bowel under study, in order that the collateral blood supply could be eliminated when desired. Shortly before the cannulas were introduced, the animals were heparinized.

The quartz rod technic[8] was used for the illumination of the blood vessels of the mesentery and a 500 Watt light source was employed. A constant gentle flow of Ringer's solution at 39 C. kept the tissues moist and warm. Motion of the tissues under study was prevented by the use of a small plastic

holder. The capillaries were observed using a binocular microscope, and from time to time were photographed on Kodachrome film employing a Kodak special movie camera. The rate of blood flow of the bowel segment was determined by temporarily disconnecting the venous cannula and timing the flow of blood. The oxygen content of the arterial and venous blood was determined by the method of Van Slyke and the rate of blood flow and the arteriovenous oxygen difference were used in computing the oxygen consumption. Pressures in the mesenteric artery and vein were determined by means of small sidearms on the cannulas and a Sanborn Electromanometer.

With the cannulas in place as shown in figure 1, studies were made with the blood flow in the normal direction, that is, from femoral artery to mesenteric artery through the capillaries and returning through the mesenteric and femoral veins. Following this, a retrograde perfusion was produced by attaching the arterial inflow cannula to the mesenteric vein, and the outflow cannula to the mesenteric artery. Under this latter condition, the arterial blood was directed at arterial pressure into the venous end of the capillary and was drained away from the arterial end of the bed. Thus the capillary bed of an area was studied with the flow directed in the normal direction or in the reverse direction, and the direction of flow was altered as desired.

Results

Thirty-one experiments were performed on 19 cats, 3 dogs, and 3 rabbits. Excellent forward capillary blood flow was observed in twenty-seven experiments during the time that the flow was in the normal direction and in seventeen of these good reversal of capillary flow was seen when the inflow was reversed. This reversal of capillary flow was demonstrated both by direct microscopic observation and by motion pictures. Failure of the capillary flow to reverse in ten studies was usually due to incomplete elimination of the collateral arterial inflow to the part, although in some instances there was no adequate explanation.

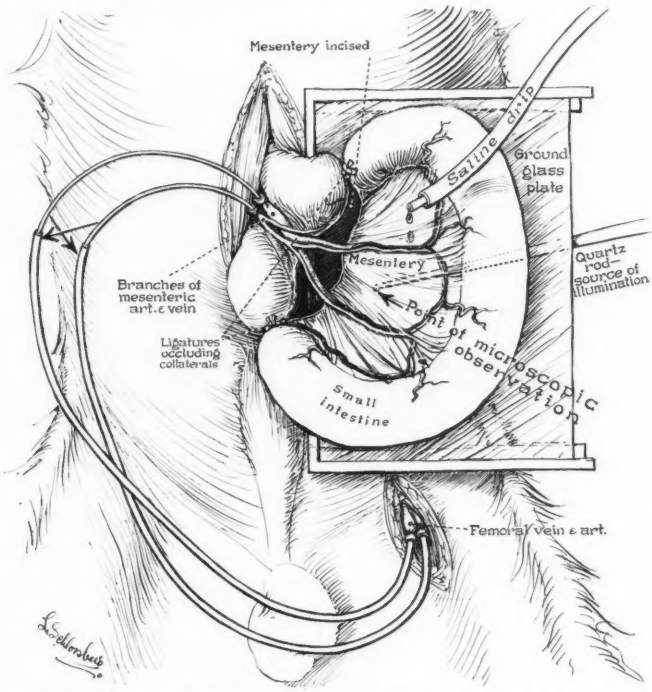


FIG. 1. Showing the experimental procedure. Part of the small intestine has been delivered through an abdominal incision. A mesenteric artery and vein have been isolated. These vessels are connected by cannulas to the femoral artery and vein. Braided silk ligatures occlude the intestines and collateral blood vessels. The capillaries of the mesentery are studied microscopically.

The longest period that reversal of blood flow was observed was 57 minutes.

The rate of flow and the oxygen consumption of the segment of intestine were determined in ten experiments. The average flow was 4.42 cc. per minute with forward flow, and 3.07 cc. per minute with reverse flow. The oxygen consumption was 0.3 cc. per minute with forward flow and 0.17 cc. per minute with reverse. These figures are given in table 1. Even gross observation of the returning blood after reverse perfusion showed that it had lost oxygen.

TABLE 1.—*Capillary Studies*

	Normal perfusion	Reverse perfusion
Excellent Microscopic Capillary Blood Flow	27 exps.	17 exps.
Average Rate of Blood Flow cc./min.	4.42	3.07
Average Tissue Oxygen Consumption cc./min.	0.3	0.17

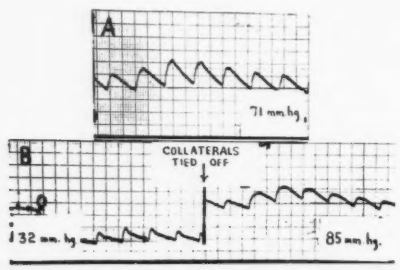


FIG. 2. Showing the effect of collateral pathways on perfusion pressure. *A*. Pressure in mesenteric artery with flow in normal direction, collaterals open. *B*. Pressure of inflowing blood in mesenteric vein with retrograde flow, collaterals open, followed by elevation in inflow pressure that occurs when collaterals are tied.

It should be stated that the cannulas offered some resistance to the flow of blood. The mean pressure in the arterial cannulas fell from 118 to 80 mm. Hg from its proximal to distal end. The venous cannulas had a pressure gradient of about 6 mm. Hg. It is felt, however, that the inflow pressures were sufficient for adequate perfusion. However, the presence of the collateral arterial supply inhibits reversal of capillary blood flow probably by causing back pressure upon the arterial side of the capillary. The effects of opening and closing the collateral pathways on the perfusion pressure are shown in figure 2.

DISCUSSION

Two other types of experiments will be commented upon briefly. An attempt was made to produce a chronic reversal of flow in a segment of bowel of heparinized animals but either death occurred or thrombosis at the site of the inflow cannula took place. In other animals, the terminal aorta and the inferior vena cava were severed, the proximal end of the aorta was anastomosed to the distal end of the vein and the distal end of the aorta to the proximal end of the artery but death occurred within 24 hours. We are not certain as to the cause of death.

Transient reversal of the direction of capillary blood flow has been noted previously by several observers. Chambers and Zweifach[9] reported this phenomenon in the normal capillary bed as a result of a rhythmic variation in the amount of entering blood. Bigelow, Heimbecker and Harrison[10] reported reversal of blood flow in capillaries due to alterations in hemodynamics associated with intravascular agglutination of blood. The present experiments show that at least temporary retrograde flow in the capillary bed can be produced by transposing the arterial inflow and the venous outflow. However, the studies indicate that this retrograde flow occurs only if the collateral arterial pathways are closed. This finding may be due in part to the resistance to inflow imposed by the cannulas. The presence of a plexus of collateral veins provides a shunt mechanism through which the blood in a mesenteric vein at an artificial arterial pressure can escape directly into the general venous return, thereby avoiding the capillary bed. The studies reported in figure 2 support this statement. At any rate, this finding in studies on the intestinal tract suggests that the presence of uninterrupted collateral arteries and veins in the heart, brain, or extremity would exert the same inhibitory influence upon the reversal of capillary blood flow. Moreover, if reversal is to occur in these organs, the blood must leave by way of the organs' arteries.

The tissue oxygen consumption fell from an average of 0.3 cc. per minute during normal perfusion to an average of 0.17 cc. during retrograde perfusion. However, in three experiments there was no decline. The figures should not be compared too critically for it should be remembered that a mesenteric artery and its adjacent vein do not necessarily connect with identical areas of the capillary bed. The figures do show that the tissues will take up oxygen from blood flowing through its capillaries in a reverse direction.

Summary

It has been shown in acute experiments on cats, dogs and rabbits that a reversal of blood flow in mesenteric capillaries will occur following the connection of an artery to a vein providing the collateral arteries and veins are obstructed. The blood must be permitted to leave the area by means of the mesenteric arteries. Blood flowing in a reverse direction through capillaries loses oxygen.

REFERENCES

[1] Roberts, J. T., Browne, R. S., and Roberts, G.: Nourishment of the myocardium by way of the coronary veins. Federation Proc. **2:** 90, 1943.

[2] Beck, C. S.: Revascularization of the heart. Surgery **26:** 82, 1949.

[3] Stenstrom, J. D.: Vascularization of the myocardial capillary bed by arterialization of the cardiac veins: an experimental study. Canad. M. A. J. **59:** 420, 1948.

[4] Johns, T. N., Sanford, M. D., and Blalock, A.: An experimental study of the anastomosis of arteries to the coronary sinus of the dog. Bull. Johns Hopkins Hosp. **87:** 1, 1950.

[5] Sciaroni, G. H.: Reversal of circulation of the brain. Am. J. Surg. **76:** 150, 1948.

[6] Beck, C. S., McKhamm, C. F., and Belnap, W. D.: Revascularization of the brain. Read before Symposium of Cardiovascular Research, U. S. Public Health Service, Washington, D. C. Jan. 21, 1950.

[7] Johnston, C. G., and Jordan, P., Jr.: Clinical use of arterio-venous shunts. Read before Symposium of Cardiovascular Research, U.S. Public Health Service, Washington, D. C. Jan. 21, 1950.

[8] Knisely, M. H.: An improved quartz rod for living tissue illumination. Anat. Rec. **71:** 503, 1938.

[9] Chambers, R., and Zweifach, B. W.: Topography and function of the mesenteric capillary circulation. Am. J. Anat. **75:** 173, 1944.

[10] Bigelow, W. G., Heimbecker, R. O., and Harrison, R. C.: Intravascular agglutination (sludged blood) vascular stasis and the sedimentation rate of the blood in trauma. Arch. Surg. **59:** 667, 1949.

Great Toe Calorimetry in Peripheral Vascular Diseases

By MILTON MENDLOWITZ, M.D., AND HAROLD A. ABEL, M.D.

Blood flow was measured in the great toe calorimetrically in various peripheral vascular diseases, after release of sympathetic nerve tone by indirect heating supplemented by tetraethylammonium chloride. There was a striking decrease in blood flow in thromboangiitis obliterans.

THE DIGITAL calorimetric technic for measuring blood flow has recently been evaluated critically and found to be sound when employed after release of sympathetic nerve tone.[1] The two most important sources of error are the possibilities that arterial blood may arrive at the digit below mouth temperature and that venous blood may leave above calorimeter temperature. The latter source of error, although important for the hand calorimeter,[1] where a considerable contribution to venous return is made by the deep tissues, has been shown to be absent in the digit especially when blood flow is measured per unit surface. When freezing calorimeter temperatures are employed, however, both venous and arterial errors become more significant and the actual blood flow is probably greater than that calculated from calorimetric data.[2] In these studies calorimeter temperatures were always about 31.0 C. Moreover, after release of sympathetic nerve tone the temperature of arterial blood was found in average to be 0.7 C. below mouth temperature,[1] a correction factor incorporated in the calculations. It is possible that at low rates of flow arterial blood temperature falls below this level, although this has not as yet been demonstrated. The values presented here, therefore, represent actual blood flow determinations with the qualification that the very low readings may on further investigation prove to be somewhat higher than represented.

The calorimetric method was first adapted to the great toe in a study of patients with residua of trench foot.[3] Blood flow was determined after 45 to 90 minutes of indirect heating. In order to reduce the time of the procedure, heating has been decreased to 30 minutes or until positive heat balance and sweating are achieved. This is supplemented by the intravenous administration to the recumbent patient of 5 mg. per Kg. of tetraethylammonium chloride.

Those patients over 55 or with severe hypertension or heart disease were not given tetraethylammonium chloride. In such patients, blood flow was measured after 60 to 90 minutes of indirect heating alone. Patients were instructed to rest for one hour after the test and not to drive vehicles during the day of the test. In a series of approximately one thousand determinations in which tetraethylammonium chloride was used in this manner, the only untoward reaction was transient vomiting in 2 patients. Indirect heating and tetraethylammonium chloride were somewhat more effective in blocking vasoconstrictive "break through" of psychic and reflex origin than indirect heating alone. Indirect heating supplemented by tetraethylammonium chloride has been demonstrated to be as effective as spinal anesthesia in releasing sympathetic nerve tone if the calorimetric method is used.[4] The normal range of variation of blood flow in the toe with this procedure was 0.15 to 0.29 cc. per cm.^{2} per minute. The average was 0.21 cc. per cm.^{2} per minute $\pm$ a standard deviation of 0.041. This is nearly the

From the New York Regional Office, Veterans Administration and the Medical Service of Dr. George Baehr, The Mount Sinai Hospital, New York, N. Y. Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the results of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

One of us (M.M.) was aided by grants from the American Heart Association and the Research Committee of the American Medical Association.

same distribution as was obtained using prolonged indirect heating alone to release sympathetic nerve tone.[3]

It has recently been shown in dogs[5] that tetraethylammonium chloride in large doses, in addition to blocking sympathetic nerve impulses, may release vasoconstrictive substances from the adrenal medulla and the liver which counteract its vasodilating action on nerves. In view of the correspondence between results with prolonged indirect heating alone and indirect heating supplemented by tetraethylammonium chloride in the dosage used, it is unlikely that this factor is of importance in these studies.

Blood flow measurements were made in 800 additional cases of trench foot. Inasmuch as the results in these cases were in accord with those already reported,[3] they will not be considered in detail in this communication. Studies were also completed in 35 cases of thromboangiitis obliterans, 5 cases of peripheral arteriosclerosis, 6 cases of Raynaud's disease, 1 case of scleroderma, 5 cases of thrombophlebitis and 3 cases of acrocyanosis. The results in thromboangiitis obliterans are analyzed statistically in table 1.

Of the 35 cases of thromboangiitis obliterans the diagnosis was definite clinically and from oscillometric data in 17 and presumptive in the remaining 18 cases. Blood flow was below the lower limit of normal in all but one of the definite group and in 13 of the 18 cases in the presumptive group. In peripheral arteriosclerosis well established clinically and by objective tests including oscillometry and roentgenographic demonstration of peripheral arterial calcification, blood flow was below the lower limit of normal in 4 of the 6 cases. Blood flow was below the normal range in 4 of the 6 cases of Raynaud's disease and in the single case of scleroderma studied, and was within the normal range in 4 of the 5 cases of thrombophlebitis and in the 3 cases of acrocyanosis. Since digital blood pressures, especially diastolic, are often inaccurate in the great toe, digital vascular resistance could not be studied and the difference in the pressure-flow pattern in large as against small arterial obstruction could not be as well defined as in the fingertip.[6]

Great toe calorimetry is useful as a diagnostic test in doubtful cases of peripheral vascular diseases and as a measure of the degree of impairment of the circulation in the great toe in established cases. It should be of value in following the effects of therapy in various types of vascular disease.

TABLE 1. *Statistical Analysis of Data*

	No. of cases	Calorimetric Blood Flow in cc./cm.²/min.					
		Range	Mean $\bar{X}$	Standard Deviation σ	Standard error of mean $\sigma_{\bar{X}}$	Standard Deviation of difference of means $\sqrt{(\sigma_{\bar{X}'})^2+(\bar{X}'')^2}$	Significance Ratio $\frac{\bar{X}'-\bar{X}''}{\sqrt{(\sigma_{\bar{X}'})^2+(\sigma_{\bar{X}''})^2}}$
Normal	24	0.15–0.29	0.21	0.041	0.009		
Thromboangiitis obliterans	35	0.01–0.23	0.10	0.047	0.008	0.012	9.2

SUMMARY AND CONCLUSIONS

Blood flow measured calorimetrically in the great toe after release of sympathetic nerve tone by indirect heating supplemented by tetraethylammonium chloride was usually below normal in peripheral arteriosclerosis, scleroderma and Raynaud's disease and usually within normal limits in thrombophlebitis and acrocyanosis. The number of cases in each of these groups, however, was too small for statistical conclusions. In thromboangiitis obliterans there was a statistically valid striking decrease in blood flow.

ACKNOWLEDGMENT

The advice of Dr. S. Feitelberg and the technical assistance of Mr. G. Grossinger, Mr. R. E. Schwartz, and Miss S. Lichtenberg are gratefully acknowledged. We are also indebted to Dr. S. Silbert for some of the cases studied.

REFERENCES

[1] MENDLOWITZ, M.: Observations on the calorimetric method for measuring digital blood flow. Angiology **1:** 247, 1950.

[2] GREENFIELD, A. D. M., AND SHEPHERD, J. T.: A quantitative study of the response to cold of the circulation through the fingers of normal subjects. Clin. Sc. **9:** 323, 1950.

[3] MENDLOWITZ, M., AND ABEL, H. A.: Quantitative blood flow measured calorimetrically in the human toe in normal subjects and in patients with residua of trench foot and frostbite. Am. Heart J. **39:** 92, 1950.

[4] —, TOUROFF, A. S. W., AND ABEL, H. A.: The effect of venous and arterial occlusion and sympathetic nerve tone on digital blood flow. J. Clin. Investigation. **30:** 94, 1951.

[5] PAGE, H., PRINCE, R., AND REINHARD, J. J.: Mechanism of the vascular action of teraethylammonium chloride. Am. J. Physiol. **158:** 403, 1949.

[6] MENDLOWITZ, M.: The digital circulation in peripheral vascular diseases. J. Clin. Investigation **21:** 547, 1942.

CLINICAL PROGRESS

Editor: HERRMAN L. BLUMGART, M.D.

Associate Editor: A. STONE FREEDBERG, M.D.

Angiocardiography

By CHARLES T. DOTTER, M.D., AND ISRAEL STEINBERG, M.D.

ANGIOCARDIOGRAPHY constitutes the roentgen study of the chambers of the heart and associated great blood vessels during their opacification by an intravenously injected radiopaque solution. The procedure as it is known today was first successfully accomplished on Jan. 30, 1937 by Robb and Steinberg.[1] During the 14 years since that time an estimated 10,000 examinations have been performed throughout the world. Accumulated experience has demonstrated its usefulness as a diagnostic examination within a continuously widening range of application.[2]

METHOD

The fundamental steps in the technic of angiocardiography may be briefly stated.[3] Employing local anesthesia, a large bore needle with an attached stopcock is inserted through a nick in the skin into an antecubital vein. An appropriate dose of contrast substance is then rapidly injected (in one to one and one-half seconds) into the vein during inspiration and films made at appropriate or multiple intervals thereafter. The contrast media in general use in this country are Neo-iopax 75 per cent and Diodrast 70 per cent, the adult dosage ranging between 40 and 50 cc. per injection. Injections may be repeated once if necessary. The projection employed and the time for x-ray exposure (if serial roentgenography is not available) depend upon the structures to be visualized.[4] The immediate subjective reaction to the injection consists of a hot flush, a feeling of weakness and sometimes a throbbing headache. Vomiting, urticaria and syncope are occasionally unpleasant but not serious side effects, while chemical thrombosis of the injected vein is usual. The procedure is not without danger; at least 26 deaths have followed, mostly in children with serious congenital cardiovascular anomalies.[5]

During recent years, many methods for automatic serial roentgenography have been employed to obtain progressive visualization of the contrast substance in its passage through the veins, the right heart, the pulmonary vessels, the left heart and the aorta. Speeds of up to twelve exposures a second in two projections simultaneously are a practical reality[6] although one or two per second exposure frequencies are usually diagnostically adequate. A commercially obtainable x-ray roll-film magazine affords 9½ inch square exposures at a frequency of up to two films per second and has proved to be a satisfactory means of angiocardiographic recording.[7, 8]

NORMAL ANGIOCARDIOGRAM

Angiocardiography has significantly added to the understanding of conventional chest roentgenography and fluoroscopy and has been of value in the teaching of radiology and anatomy.[9] Its ability to provide a gross "dissection" of the heart and mediastinum during life is unique among the varied methods of cardiovascular study. Angiocardiography has provided confirmation of certain assumptions concerning the composition of the cardiac silhouette; it has dispelled other erroneous concepts. For example, while verifying the predominantly vascular composition of the normal hilar shadows, angiocardiography has shown conclusively that the pulmonary conus does not participate in the formation of the heart shadow in the frontal projection. Figure 1 illustrates the composition of the normal cardiac silhouette in

From the Department of Radiology of The New York Hospital-Cornell Medical Center.

This investigation was aided by grants from the Schering Corporation and The New York Heart Association.

postero-anterior projection as it is revealed angiocardiographically. By defining the normal limits of diameter and length of the great blood vessels, angiocardiography has made possible the recognition and definition of abnormal anatomy. It is anticipated that further painstaking and carefully controlled measurement studies will eventually define the limits of the normal cardiac chambers in terms of diameters and volumes related to different phases of the cardiac cycle under varied hemodynamic situations.

gained is necessary for formulating a therapeutic approach.

Coarctation and Other Anomalies of the Aorta

In revealing the abnormal anatomy present, angiocardiography is a definitive preoperative means of diagnosis in coarctation of the aorta. Surgically significant differences in the site of the stenosis are compatible with identical clinical findings. Angiocardiography is best performed in the left anterior oblique projection (the view presented to the surgeon at thoracotomy). The site of coarctation is generally 1 or 2 cm. distal to the origin of the left subclavian artery, the length of the intervening stub often being the determining factor in the choice of operative procedure. The collateral vessels are usually well shown and dilatation of the ascending aorta is common (fig. 2).

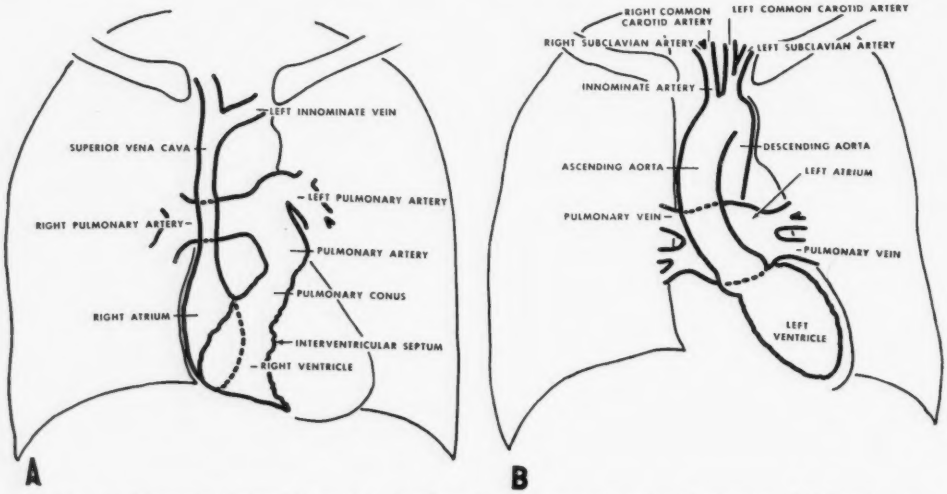


FIG. 1. *Normal Angiocardiographic Configuration, Postero-Anterior Projection.* Idealized diagrams representing structures as seen during ventricular diastole. *A*. Superior vena cava, right heart and pulmonary arteries at about 2.5 seconds after beginning of injection. *B*. Pulmonary veins, left heart and aorta at about 8 to 10 seconds.

CONGENITAL HEART DISEASE

It is in the field of congenital heart disease that angiocardiography has been most widely applied and most persistently abused. The nature of the procedure makes it ideal for the investigation of certain lesions such as coarctation of the aorta while, contrary to general belief and usage, it is of limited value in the study of other defects such as those producing left to right shunts. The selection of cases for angiocardiography, cardiac catheterization or both procedures should be made after complete clinical evaluation. Such studies are not without danger and should be reserved for those cases wherein the information to be

Angiocardiography demonstrates the anatomy but not the etiology of congenital aneurysms of the aortic arch and is often of value in distinguishing between such lesions and mediastinal tumors in young patients.[10] In the presence of congenital aortic or subaortic stenosis, visualization may reveal a dilatation or irregularity of the ascending aorta.[11]

Anomalies in the course of the aortic arch and its branches are accessible to angiocardiography although conventional methods of diagnosis are in most cases reliable.

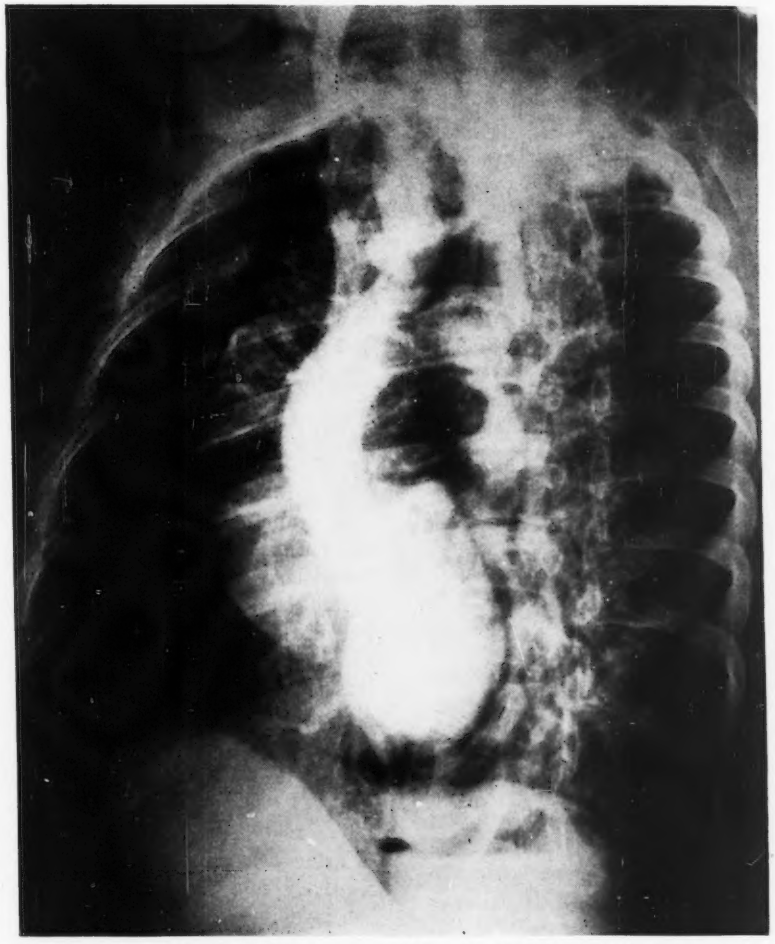


FIG. 2. *Coarctation of Aorta.* Thirty-two year old female (N.Y.H. 440 369). Left anterior oblique angiocardiogram at 6.5 seconds. The point of coaretation is visualized 1.5 cm. distal to the left subclavian artery. Dilated innominate, subclavian and internal mammary arteries participate in collateral circulation. Anatomic information such as is shown in this film is of great value in the preoperative decision as to whether or not an aortic graft will be necessary.

Left-Right Shunts

Intracardiac left to right shunts such as those produced by isolated interatrial or interventricular defects are best diagnosed by clinical means or cardiac catheterization. The characteristic combination of marked right heart enlargement and pulsating dilated pulmonary arteries is more reliable evidence of interatrial septal defect than is the usual angiocardiographic finding of re-opacification of the right atrium, the right ventricle and the pulmonary arteries at the time of aortic filling. In young children, contrast substance may occasionally be seen passing directly from the right to the left atrium. Isolated ventricular septal defects are demonstrable by angiocardio-

graphy in few instances and the findings are rarely if ever decisive. Patent ductus arteriosus may be suspected upon the basis of re-opacification of the pulmonary arteries or the demonstration of a localized dilatation of the aorta at the site of origin of the ductus[12] but such evidence is not as reliable as that obtained by the use of a stethoscope. Aortic septal defect may mimic patent ductus arteriosus even after angiocardiography and cardiac catheterization but may be definitively diagnosed by retrograde aortography.

Isolated Pulmonary Stenosis

Uncomplicated pulmonary stenosis is frequently responsible for marked dilatation of the pulmonary artery distal to the site of narrowing. Contrast visualization reveals the dilatation but not invariably its cause. If a site of narrowing is not revealed, cardiac catheterization pressure studies should be conducted in order to distinguish between poststenotic dilatation and that due to other congenital lesions such as primary congenital pulmonary artery aneurysm. Angiocardiography may establish but never exclude the diagnosis of pulmonary stenosis.

Anomalous Pulmonary Veins

Contrast visualization is unique in its ability to demonstrate the anatomy of anomalous pulmonary veins entering the right atrium or its immediate tributaries.[13] If the abnormal drainage is partial, the lesion is compatible with normal existence although in effect constituting a "physiologic pneumonectomy." Although cardiac catheterization findings may suggest the presence of an interatrial defect (if the catheter does not enter the anomalous vessel), angiocardiography leaves no doubt as to the diagnosis.

Cyanotic Congenital Heart Disease

Candidates for the operative alleviation of congenital pulmonary stenosis should be routinely studied angiocardiographically in order to minimize diagnostic error and indicate the proper operative approach. The surgeon, able to make only a limited inspection of a rapidly moving heart during thoracotomy, deserves all available anatomic information.

It is present-day practice to attempt the classification of congenital cyanotic malformations into a variety of pathologic entities such as tetralogy of Fallot, pseudo-truncus arteriosus or pulmonary artery atresia. Although pathologically and embryologically sound, it is frequently difficult to perceive any physiologic or surgical significance to such distinctions (which cannot usually be made during life). Angiocardiographic interpretation is most profitably directed toward the identification of specific lesions such as the presence and degree of over-riding of the aorta and the state of the pulmonary circulation.[14] Angiocardiography can be relied upon to show gross reduction in pulmonary blood flow and usually indicates the route by which blood reaches the lungs (i.e. through dilated, normal or stenotic pulmonary arteries, bronchial arteries or patent ductus arteriosus). The interpretation of angiocardiograms is best undertaken by those conversant with the clinical and laboratory data. The knowledge of a left axis deviation coupled with cyanosis immensely facilitates the identification of hypoplastic or nonfunctioning right ventricle.[15] In the interpretation of angiocardiograms (as any laboratory data), the distinction between facts and inferences drawn from facts should be constantly recalled. Figure 3 with its legend illustrates a logical approach to angiocardiographic interpretation in complex cyanotic congenital heart disease.

Acquired Heart Disease

The role of angiocardiography in the study of arteriosclerosis and hypertension has been relatively minor and primarily academic. It has made possible an evaluation during life of the aortic changes produced by these diseases. Hypertension causes moderate dilatation of the ascending aorta, arteriosclerosis is chiefly manifested by elongation.[16]

In our experience, dilatation of the ascending aorta of above 40 mm. caliber is rarely if ever due to arteriosclerosis alone, although it may be the result of hypertension, syphilis or aortic insufficiency. Angiocardiography has demonstrated dilatation of the superior vena cava, congestion of the pulmonary vessels and delayed circulatory rate in heart failure.[2] It has

assisted in the localization of foreign bodies in or related to the heart.[17] The method has proved to be extremely productive as a diagnostic tool in certain forms of acquired heart disease.

Syphilis of the Aorta

Since the introduction of angiocardiography, its ability to distinguish with certainty between aneurysm and mediastinal tumor has been repeatedly stressed and need not be further emphasized. The procedure has proved to be of great value in the detection of uncomplicated syphilitic aortitis by the demonstration of the following abnormalities[18]: (1) dilatation of the mid-ascending aorta beyond the limit of 38 to 40 mm. in the absence of nonsyphilitic cause for such dilatation (hypertension, aortic insufficiency, congenital anomalies involving the aorta); (2) irregularity of the aortic lumen; (3) variations in thickness of the aortic wall; (4) tortuosity of the aorta. By angiocardiography, the diagnosis of syphilitic aortitis has repeatedly been made in the absence of demonstrable clinical or conventional roentgenologic abnormality.[19]

Rheumatic Heart Disease

Angiocardiography has played a significant role in the demonstration of the anatomic changes responsible for the characteristic cardiac silhouette in mitral stenosis. The prominence in the left midcardiac border as seen in frontal study is caused by the dilated left auricular appendage and dilated pulmonary artery.[20] It is not the pulmonary conus. The prominence of the anterior heart border seen to encroach upon the retrosternal space in the lateral chest roentgenogram of patients with mitral stenosis has been widely attributed to dilatation and hypertrophy of the right ventricle and its pulmonary conus. Contrast visualization (fig. 4) has shown that this abnormality is largely the result of left atrial dilatation, the latter causing anterior displacement of the superior vena cava and the right heart chambers.[2]

Pericardial Effusion

By virtue of its ability to reveal the limits of the cardiac chambers, angiocardiography affords an accurate means of demonstrating the presence and estimating the amount of pericardial effusion.[21] In the presence of effusion, an abnormally wide interval (greater than 0.5 cm.) is seen to exist between the opaque substance in the right atrium and the margin of the cardiac silhouette. Similar extracardiac density lateral to the left ventricle is confirmatory. Angiocardiography has shown the absence of pericardial effusion in the presence of a classic "low voltage" electrocardiogram due to myxedema heart and conversely, in another patient with myxedema, has shown the presence of an effusion in the absence of electrocardiographic change.

Ventricular Aneurysm

Aneurysm of the left ventricle is occasionally difficult to distinguish from tumors of or adjacent to the heart. The angiocardiographic demonstration of opacification of the lumen of the aneurysm is diagnostically decisive in such instances.

Dissecting Aneurysm

In dissecting aneurysm of the aorta, angiocardiography usually shows abrupt narrowing of the aortic lumen at the site of the dissection associated with sudden increase in thickness of the aortic wall.[22] The fact that contrast substance is rarely seen within false channels is explained on the basis of previous clot formation or the presence of insufficient opaque material to cast a recognizable image on the film. Dilatation of the ascending aorta is usually seen in the presence of dissection in the distal arch and is thought to be the result of hypertension acting upon the pathologically weak aortic wall (fig. 5).

Pulmonary and Mediastinal Tumors

The conventional roentgenographic examination of mediastinal or pulmonary tumors is largely limited to the delineation of portions of masses protruding into lung fields or deforming identifiable parts of the trachea or esophagus. By means of angiocardiography, borders of tumors in contact with the chambers of the heart and great mediastinal vessels may be shown. Of primary importance has been the demonstration of the nature of the vascular

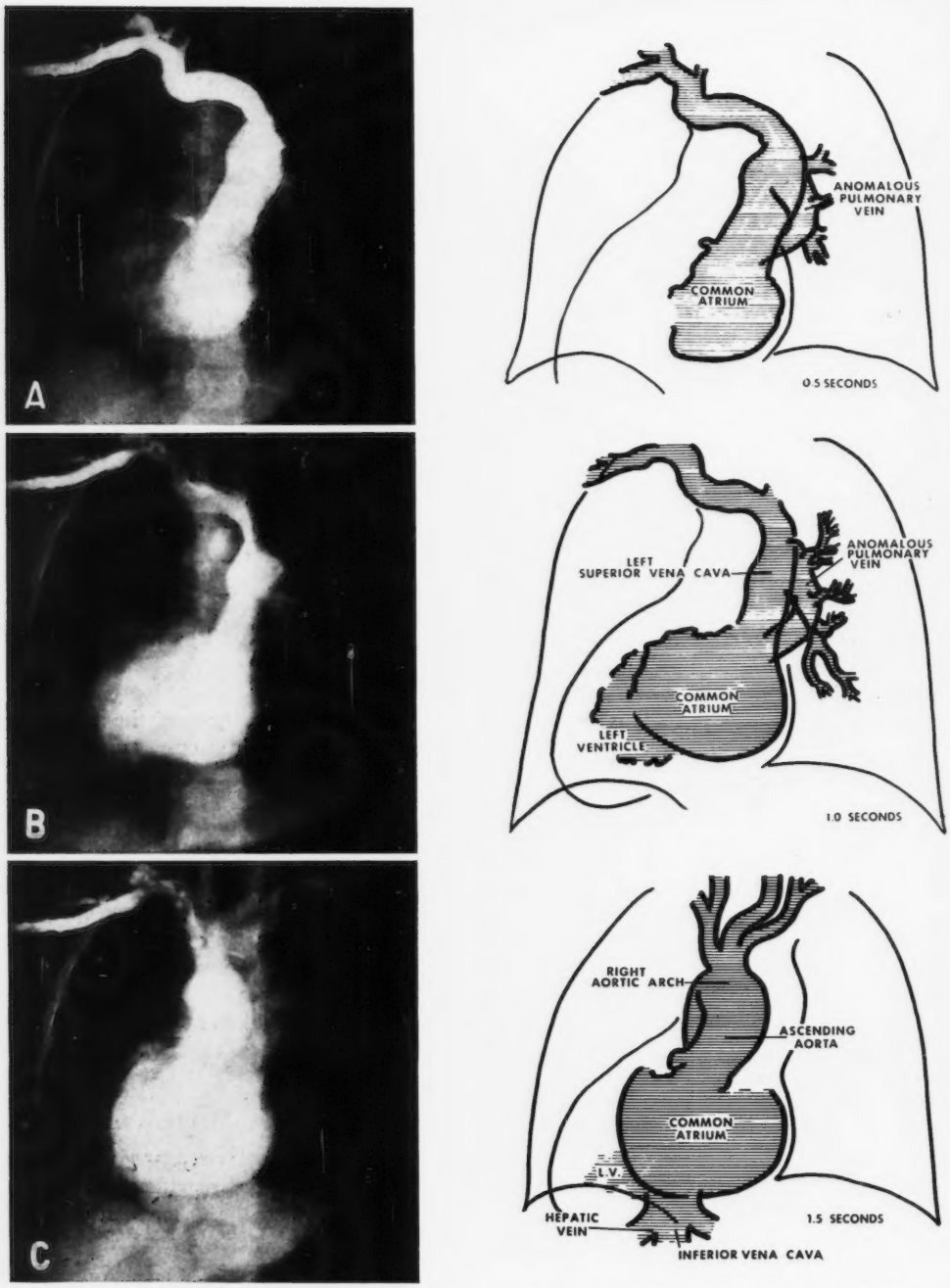
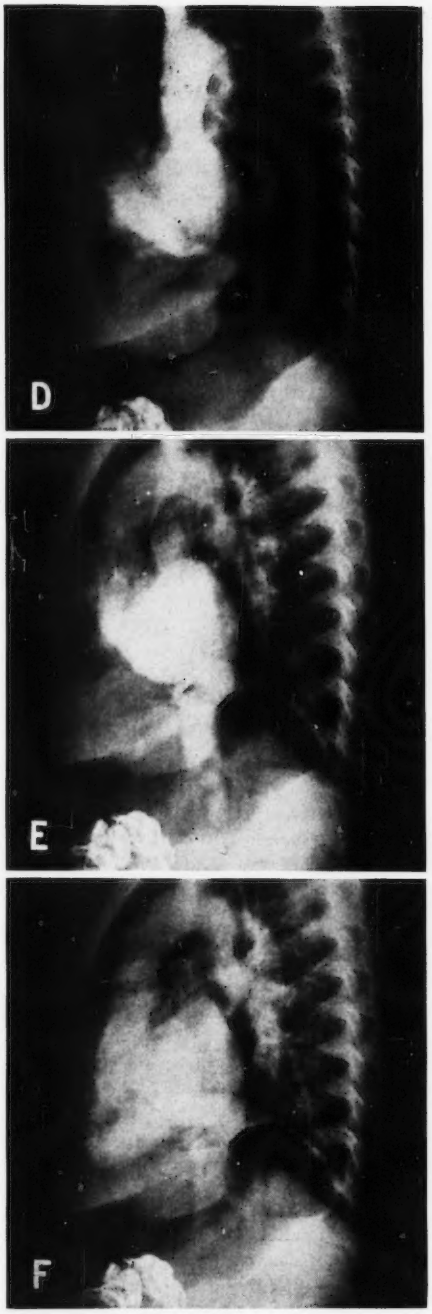


FIG. 3. *Complex Cyanotic Congenital Cardiovascular Malformation.* Four year old male (N.Y.H. 576 065), cyanotic since 6 months of age, showing left ventricular hypertrophy on unipolar electrocardiograms. Angiocardiograms done with two injections of 20 cc. 75 per cent Neo-iopax under ether anesthesia. Films made on Fairchild magazine at 0.5 second intervals. *A.*, *B.*, *C.* At 0.5, 1.0 and 1.5 seconds after injection, frontal projection. Films reveal an anomalous left pulmonary vein (filled refluxly) draining into a persistent left superior vena cava which enters a common atrium. Only one ventricle is seen, this giving rise to a large aorta which arches to the right. Pulmonary arteries not



shown. *D.*, *E.*, *F.* at 1.0, 1.5 and 2.0 seconds in lateral projection. The anomalous pulmonary vein is again shown as is the common atrium and a single large ventricle (in systole in *E*). The aorta arises far anteriorly and gives rise to a large patent ductus arteriosus through which blood enters the pulmonary arteries. *Diagnosis: Tricuspid atresia, nonfunctioning right ventricle, pulmonary stenosis or atresia, common atrium, anomalous left pulmonary vein entering left superior vena cava, dextrocardia with right aortic arch.* Save for the inferred diagnosis of tricuspid atresia, all of the lesions are directly demonstrable on the angiocardiogram.

changes produced by tumors. The contrast visualization of pulmonary vessels, particularly in the hilar areas, may influence the surgeon in deciding on the operability of a given lesion and in planning the surgical attack.[23]

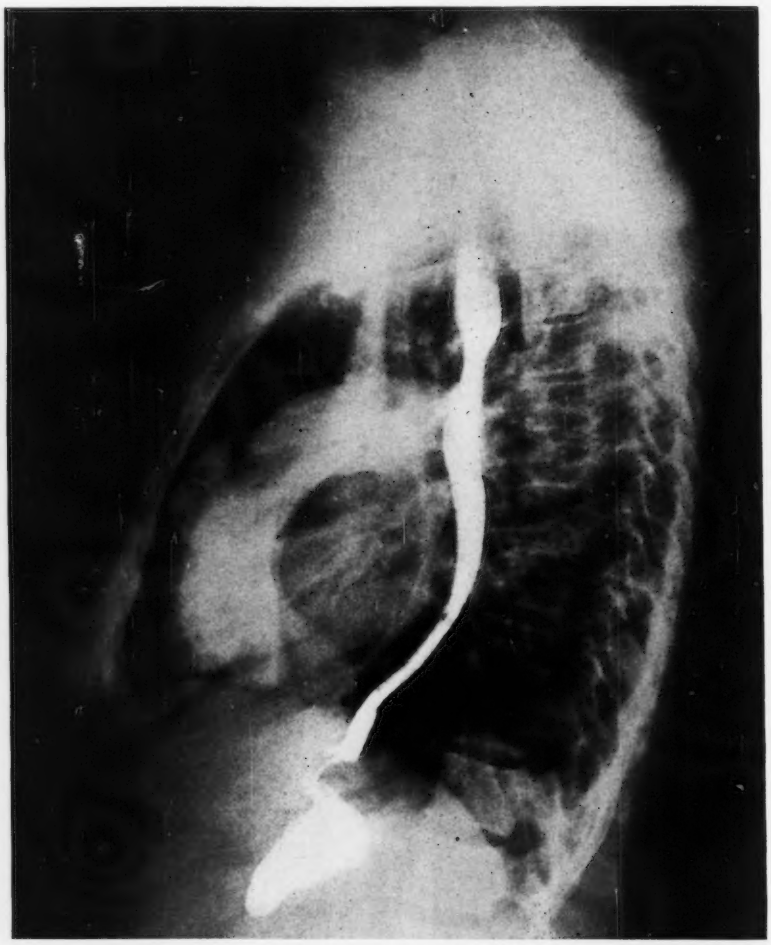


FIG. 4. *Rheumatic Mitral Stenosis.* Thirty-seven year old female (N.Y.H. 133 521) with clinical evidence of mitral stenosis. Angiocardiogram at 2.5 seconds (barium outlining esophagus) shows filling of right heart and pulmonary arteries. The enlarged left atrium has pushed the right heart forward causing increased convexity of the anterior heart border. The esophagus is similarly displaced backward. The right ventricle, pulmonary conus and arteries are not demonstrably enlarged; the left atrial enlargement is largely responsible for the abnormal cardiac silhouette.

Mediastinal Tumors

Benign mediastinal tumors as exemplified by dermoid, bronchial or pericardial cysts usually displace major vascular structures smoothly if at all. While the superior vena cava is often dislocated by bronchial cysts, it is rarely completely obstructed or the site of an irregular stenosis. The angiocardiographically demonstrable changes in vessels wrought by benign as opposed to malignant tumors may be likened to the variation in the appearance of the barium-filled stomach produced by

external pressure as opposed to intrinsic carcinoma. Unfortunately, however, the distinction is not always reliable. Retrosternal thyroid enlargement may produce a seemingly malignant stenosis of the innominate veins while a malignant mediastinal lymphoma frequently fails to produce vascular distortion. An example of the meager angiocardiographic changes produced by a thymoma is illustrated in figure 6.

A diagnosis of malignant mediastinal tumor may often be hazarded on the basis of angiocardiographic changes. Superior vena caval obstruction caused by a malignant teratoma is shown in figure 7. Other equally malignant lesions may compress without invading vessels while many produce no distortion of the vascular structures which they surround. The angiocardiographic demonstration of multiple

FIG. 5. *Dissecting Aneurysm of Aorta.* Sixty-one year old female (N.Y.H. 570 721), who had an episode of sudden severe chest pain a month before angiocardiography and whose blood pressure was 168/105. Angiocardiogram in left anterior oblique projection at 13 seconds shows widening of the ascending aorta (45 mm.). Beginning at the transverse aorta, there is irregular narrowing of the aortic lumen and marked thickening of the aortic wall, the characteristic angiocardiographic findings in dissecting aneurysm.

nonvascular mediastinal masses suggests the presence of a lymphoma but similar changes may be produced by metastatic, infectious or granulomatous mediastinal lymphadenopathy.[24] In general, angiocardiography offers anatomic information which is often of value in the differential diagnosis of benign and malignant mediastinal tumors. It gives the surgeon a much needed preoperative glimpse behind the tumor he plans to attack and may in this manner help prevent inadvertent, possibly catastrophic, operative entry into important vascular structures.

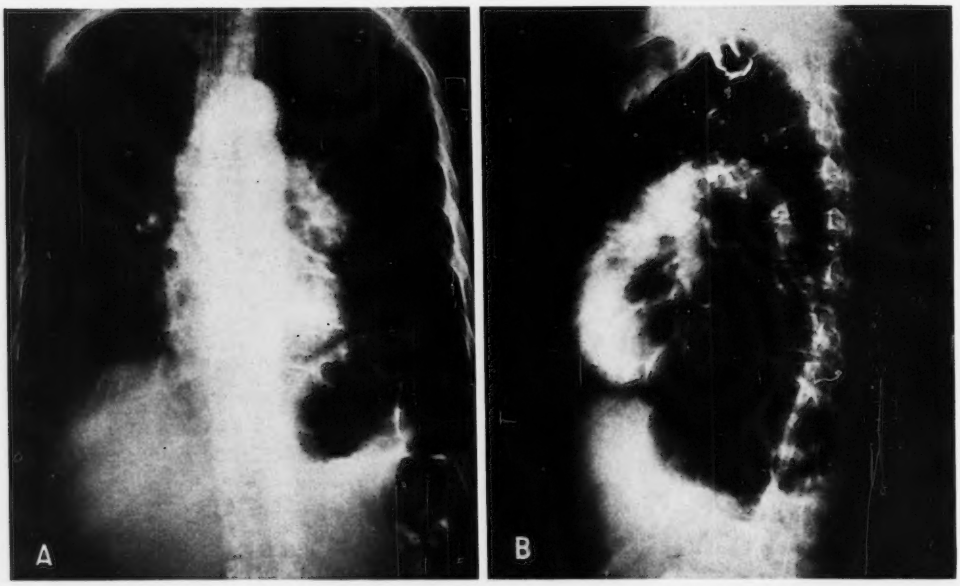


FIG. 6. *Thymoma.* Fifty-five year old female (N.Y.H. 574 356), asymptomatic. *A.* Frontal angiocardiogram at nine seconds shows normal left heart and aorta. An oval left hilar mass is not filled. *B.* Left anterior oblique projection at 2.5 seconds showing normal right heart and pulmonary arteries. The lack of vascular deformity does not rule out malignancy. At operation a thymoma was removed. Such lesions may cause much more marked displacement of vascular structures but usually fail to cause irregular stenosis or obstruction of adjacent vessels.

Cancer of the Lung

Angiocardiography often affords information of both diagnostic and prognostic significance in cancer of the lung.[25] In general, circumscribed tumors (whether benign or malignant), tend to spread or dislocate vessels rather than occlude them whereas the invasive nature of cancer causes irregular alterations in the contour of vascular lumens, frequently producing complete occlusion of major pulmonary arteries. Both infiltrative neoplasms and chronic pulmonary infections produce angiocardiographic evidence of avascularity, real or apparent, as well as distortion of the pulmonary vessels. Stenosis or occlusion must not be confused with reduced filling of pulmonary vessels.

Angiocardiography offers a means of establishing inoperability of lung cancer by demonstrating the neoplastic involvement of vascular structures beyond the limit of surgical resectability. It is to be emphasized that neoplastic invasion without vascular deformity may readily occur. Prognostic data derived from angiocardiography must be predicated upon the assumption that tumor has produced the observed changes and should therefore constitute only a part of the total evaluation of the indi-

v dual case. The most convincing angiocardiog aphic evidence of inoperability in lung cancer h is been the demonstration of partial or complete occlusion of the left pulmonary artery

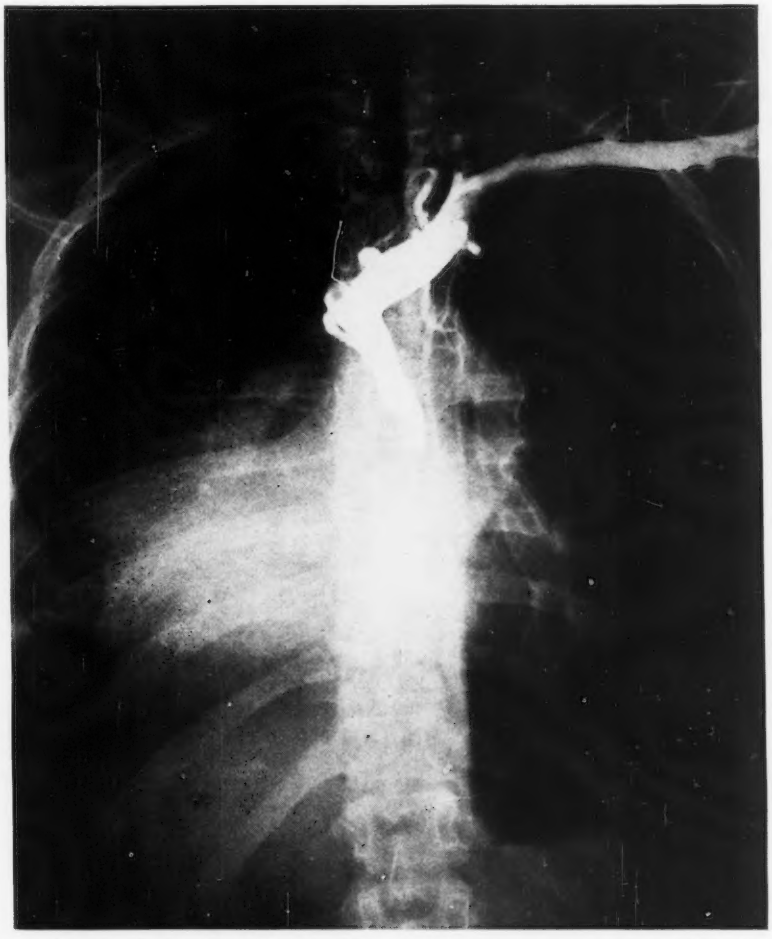


FIG. 7. *Malignant Inoperable Mediastinal Teratoma.* Twenty-two year old girl (N.Y.H. 533 051) with a two week history of cough and chest pain. Frontal angiocardiogram at 2.5 seconds. The superior vena cava is displaced medially and sharply and irregularly obstructed as though surrounded and invaded by tumor. At thoracotomy an inoperable teratoma was encountered. The angiocardiogram established the presence of tumor and strongly suggested inoperability.

at or close to its origin (fig. 8) or of the right pulmonary artery proximal to its site of bifurcation.

PULMONARY DISEASE

By virtue of the fact that the pulmonary vasculature is readily accessible to accurate visualization, angiocardiography has been extensively employed in the study of a variety of chronic pulmonary diseases. The information derived has been chiefly of academic importance in that it has supplemented data previously obtained at necropsy.

Emphysema

In the presence of localized or diffuse pulmonary emphysema, the pulmonary arteries

are seen to be widely separated in involved areas of the lung. In the case of localized bullous emphysema, angiocardiography has allowed the precise localization of involved areas

Fibrosis

Angiocardiography usually fails to demonstrate abnormal changes in the appearance of the pulmonary segmental arteries in associa-

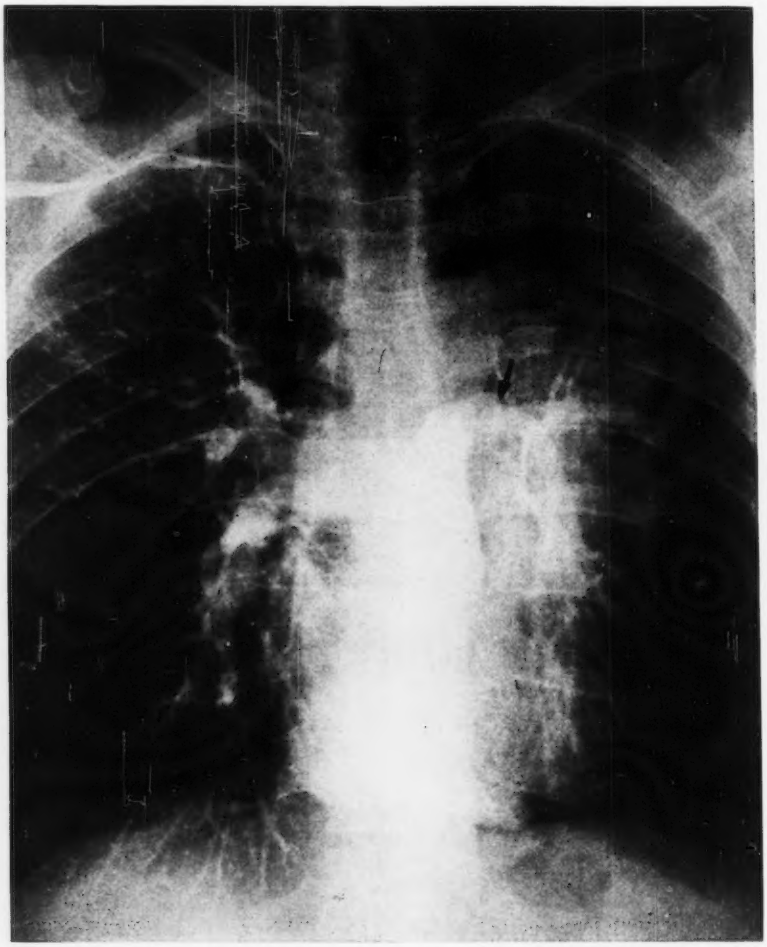


FIG. 8. *Carcinoma of the Lung.* Fifty year old male (N.Y.H. 526 450) who complained of cough, chest pain and weight loss. Frontal angiocardiogram at 3.0 seconds reveals irregular stenosis of the left pulmonary artery (arrow) in the region of a left upper lobe mass, a finding pointing toward the diagnosis of malignant pulmonary tumor. The lesion was not demonstrably inoperable by angiocardiography since there was apparently normal left pulmonary artery proximal to the involved segment. At thoracotomy a "frozen hilum" due to carcinoma of the lung was found.

of lung by study of the segmental pulmonary arteries.[26] It has given anatomic explanation to the improvement in pulmonary function that often results from resection of the involved lobe or bronchopulmonary segment (fig. 9).

tion with pulmonary fibrosis. In advanced cases the peripheral vessels may show changes consisting of alteration from normal in contour, lumen, caliber and distribution. The avascularity often encountered in the presence of a

variety of chronic pulmonary inflammatory processes is probably the result of fibrotic structures and diminished blood flow to the affected lung.

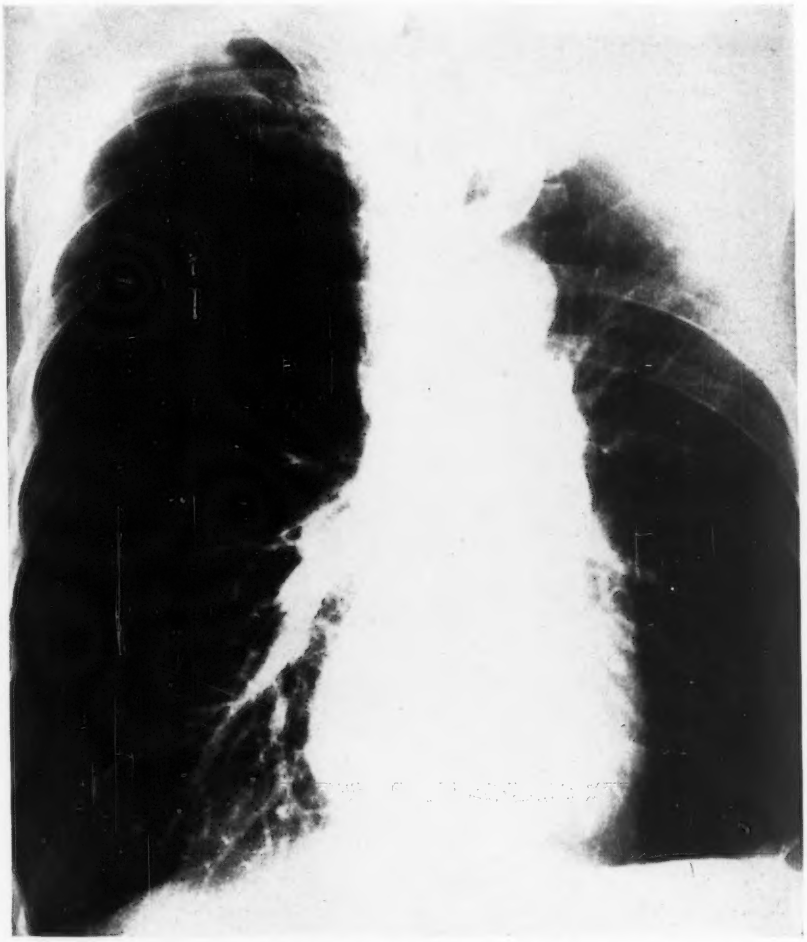


FIG. 9. *Bullous Emphysema. Postlobectomy.* Fifty-six year old male (N.Y.H. 541 430) greatly incapacitated by dyspnea until left upper lobectomy for bullous emphysema. Frontal angiocardiogram at 3.0 seconds shows markedly abnormal displacement of pulmonary arteries on unoperated (right) side in contrast to relatively normal appearing pulmonary segmental arteries to the re-expanded left lower lobe. Angiocardiography prior to operation allowed the correct prediction that functional improvement would follow resection of the left upper lobe.

change and diminished blood flow through chronically atelectatic areas of lung. In the chronic unilateral reduction of lung volume with pleural thickening, atelectasis and retraction of the mediastinum sometimes referred to as "fibrothorax," angiocardiography demonstrates altered position of cardiovascular

Tuberculosis

In no instance has angiocardiography established the diagnosis of pulmonary tuberculosis; rather, it has supplemented the information already known from pathologic study. The angiocardiographic changes in pulmonary tuberculosis include the demonstration of decreased

vascularity of involved areas probably due to thrombosis or occlusion of segmental and major pulmonary arteries.[27] The fibrosis and atelectasis incidental to longstanding or healed upper lobe tuberculosis often results in elevation and distortion of the hilar pulmonary vessels. Angiocardiography has lent support to pre-existing beliefs that pneumothorax, pneumoperitoneum, thoracoplasty and phrenic nerve crush produce varying degrees of reduction in pulmonary blood flow to the affected side.[28,29]

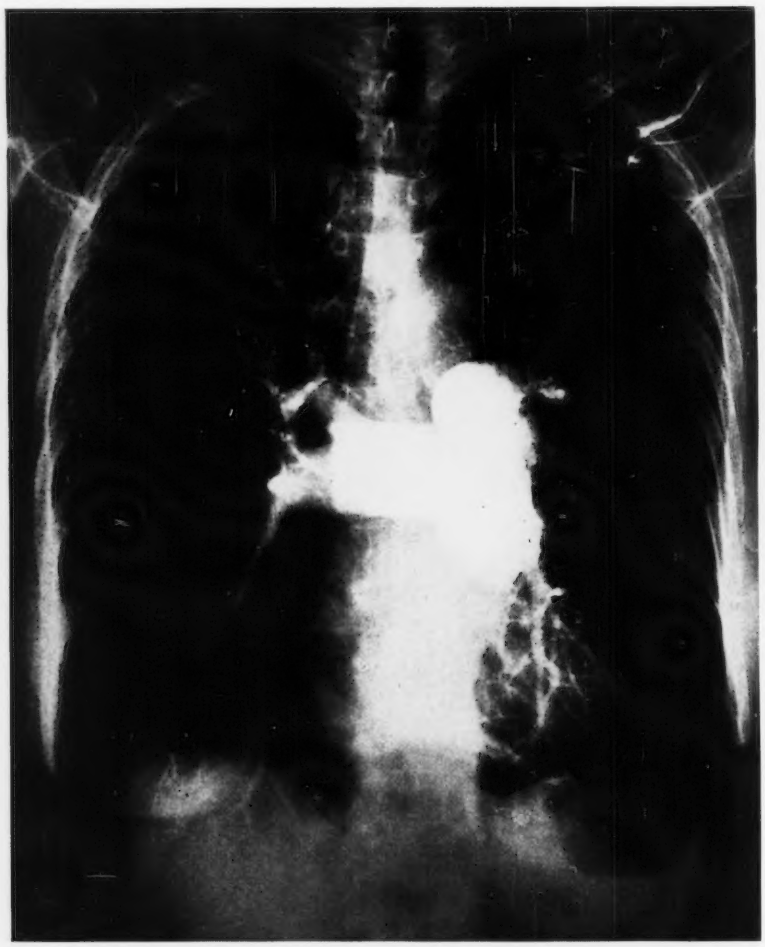


FIG. 10. *Pulmonary Heart Disease.* Fifty-two year old female (N.Y.H. 566 426) with dyspnea on exertion and evidence of severe emphysema on pulmonary function studies. At cardiac catheterization, resting pulmonary arterial pressure was 43/21 mm. Hg rising to 75/42 after exercise. Frontal angiocardiogram shows an essentially normal right ventricle, but demonstrates considerable enlargement of the central pulmonary arteries in keeping with the patient's pulmonary hypertension. Peripheral vessels show characteristic "fanning" of emphysema. Case represents extremely early pulmonary heart disease detectable only by special functional and anatomic studies.

Pulmonary Heart Disease

Angiocardiography has proved to be somewhat disappointing in the study of cor pulmonale. The angiocardiographic demonstration of slight to moderate enlargement of the right

ventricle is made with difficulty and early cases of pulmonary heart disease may show nothing more than enlarged pulmonary arteries (fig. 10). Pulmonary function studies including right heart catheterization are the most productive means of investigating pulmonary heart disease.

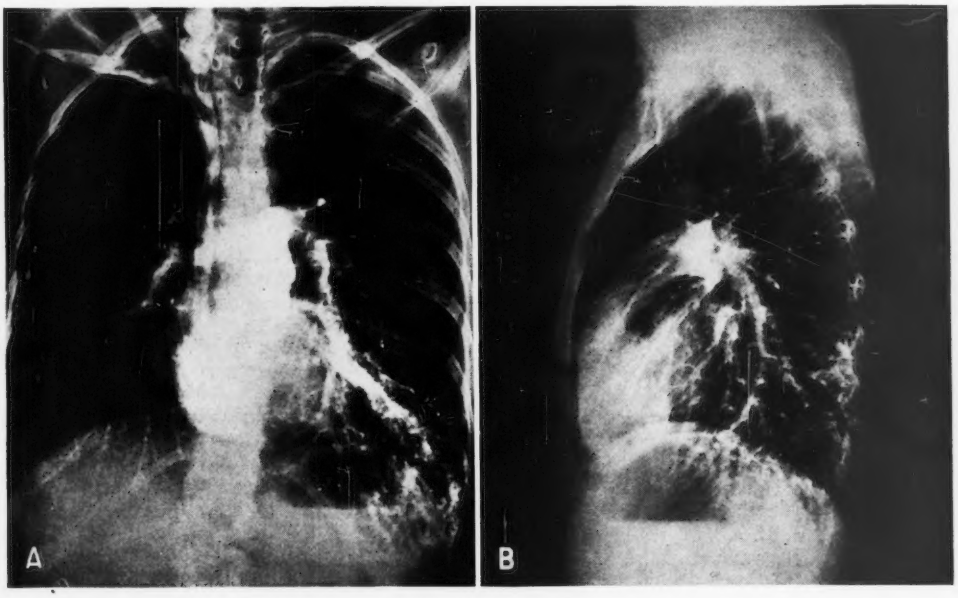


FIG. 11. *Pulmonary Arteriovenous Fistula.* 31 year old female (N.Y.H. 322 967). Clubbing, cyanosis and polycythemia. *A*. Frontal angiocardiogram at 2.5 seconds. The left pulmonary artery gives rise to numerous arteriovenous intercommunications. *B*. The lesion is seen to occupy diffusely the majority of the left lower lobe. Patient refused surgery.

Pulmonary Arteriovenous Fistula

Direct connections between pulmonary arteries and veins of congenital etiology have been the subject of a large number of recent case reports.[30] This has been in large part due to the diagnostic accuracy of angiocardiography in this condition. Films timed to show filling of the pulmonary arteries show opacification of abnormal channels or aneurysmal spaces directly connecting pulmonary arteries and veins (fig. 11). This lesion, since it shunts unoxygenated blood into the left atrium, causes cyanosis and polycythemia. Since its origin is usually presumed to be congenital, the disease is in a strict sense of the word a cyanotic congenital cardiovascular anomaly (save for the few cases resulting from trauma). Surgical resection of the involved portion of lung effects a cure.

REFERENCES

[1] ROBB, G. P., AND STEINBERG, I.: Practical method of visualizing chambers of the heart, pulmonary vessels and great blood vessels in man. J. Clin. Investigation **17:** 507, 1938.

[2] DOTTER, C. T., AND STEINBERG, I.: Clinical angiocardiography: a critical analysis of the indications and findings. Ann. Int. Med. **30:** 1104, 1949.

[3] ROBB, G. P., AND STEINBERG, I.: Visualization of the chambers of the heart, the pulmonary circulation, and the great blood vessels in man: a practical method. Am. J. Roentgenol. **41:** 1, 1939.

[4] DOTTER, C. T., AND STEINBERG, I.: Angiocardiographic interpretation. Radiology **53:** 513, 1949.

[5] —, AND JACKSON, F. S.: Death following angiocardiography. Radiology **54:** 527, 1950.

[6] FREDZELL, G., LIND, J., OHLSON, E., AND WEGELIUS, C.: Direct serial roentgenography in two planes simultaneously at 0.08 second intervals: physiological aspects of roentgen diagnosis; the apparatus and its application to angiocardiography. Am. J. Roentgenol. **63:** 548, 1950.

[7] Dotter, C. T., Steinberg, I., and Temple, H. L.: Automatic roentgen-ray roll-film magazine for angiocardiography and cerebral arteriography. Am. J. Roentgenol. **62:** 355, 1949.

[8] Scott, W. G., and Moore, S.: Rapid automatic serialization of x-ray exposures by the rapidograph, utilizing roll film nine and one-half inches wide. Radiology **53:** 846, 1949.

[9] Robb, G. P., and Steinberg, I.: Visualization of the chambers of the heart, the pulmonary circulation and the great blood vessels in man: summary of method and results. J. A. M. A. **114:** 474, 1940.

[10] Steinberg, M. F., Grishman, A., and Sussman, M. L.: Non-syphilitic aneurysm of the aorta in individuals under 45 years of age. J. Thoracic Surg. **12:** 704, 1943.

[11] Grishman, A., Steinberg, M. F., and Sussman, M. L.: Congenital aortic and subaortic stenosis with associated anomalies of the aorta. M. Clin. North America **31:** 543, 1947.

[12] Steinberg, M. F., Grishman, A., and Sussman, M. L.: Angiocardiography in congenital heart disease. III. Patent ductus arteriosus. Am. J. Roentgenol. **50:** 306, 1943.

[13] Dotter, C. T., Hardisty, N. M., and Steinberg, I.: Anomalous right pulmonary vein entering the inferior vena cava: report of two cases. Am. J. M. Sc. **218:** 31, 1949.

[14] Cooley, R. N., Bahnson, H. T., and Hanlon, C. R.: Angiocardiography in congenital heart disease of cyanotic type with pulmonic stenosis. I. Observations on the tetralogy of Fallot and "pseudo-truncus arteriosus." Radiology **52:** 329, 1949.

[15] —, Sloan, R. D., Hanlon, C. R., and Bahnson, H. T.: Angiocardiography in congenital heart disease of cyanotic type. II. Observations on tricuspid stenosis or atresia with hypoplasia of the right ventricle. Radiology **54:** 848, 1950.

[16] Dotter, C. T., Roberts, D. J., Jr., and Steinberg, I.: Aortic length. Angiocardiographic measurements. Circulation **2:** 915, 1950.

[17] —, and Steinberg, I.: Advances in angiocardiography. M. Clin. North America **34:** 745, 1950.

[18] Steinberg, I., Dotter, C. T., Peabody, G., Reader, G., Heimoff, L., and Webster, B.: The angiocardiographic diagnosis of syphilitic aortitis. Am. J. Roentgenol. **62:** 655, 1949.

[19] Peabody, G. E., Reader, G. G., Dotter, C. T., Steinberg, I., and Webster, B.: Angiocardiography in the diagnosis of cardiovascular syphilis. Am. J. M. Sc. **219:** 242, 1950.

[20] Robb, G. P., and Steinberg, I.: Visualization of the chambers of the heart, the pulmonary circulation, and the great blood vessels in heart disease. Am. J. Roentgenol. **42:** 14, 1939.

[21] Williams, R. G., and Steinberg, I.: The value of angiocardiography in establishing the diagnosis of pericarditis with effusion. Am. J. Roentgenol. **61:** 41, 1949.

[22] Golden, A., and Weens, H. S.: The diagnosis of dissecting aneurysm of the aorta by angiocardiography: report of a case. Am. Heart J. **37:** 114, 1949.

[23] Steinberg, I., Dotter, C. T., and Andrus, W. D.: Angiocardiography in thoracic surgery. Surg., Gynec. & Obst. **90:** 45, 1950.

[24] —, and Robb, G. P.: Mediastinal and hilar angiography in pulmonary disease: a preliminary report. Am. Rev. Tuberc. **38:** 557, 1938.

[25] Dotter, C. T., Steinberg, I., and Holman, C. W.: Lung cancer operability. Am. J. Roentgenol. **64:** 222, 1950.

[26] Miscall, L., Steinberg, I., and Dotter, C. T.: The surgical treatment of bullous emphysema. To be published.

[27] Steinberg, I., McCoy, H. I., and Dotter, C. T.: Angiocardiographic findings in pulmonary tuberculosis. Dis. of Chest **19:** 510, 1951.

[28] —, McCoy, H. I., and Dotter, C. T.: Angiocardiographic findings in artificial pneumothorax. Am. Rev. Tuberc. **62:** 350, 1950.

[29] McCoy, H. I., Steinberg, I., and Dotter, C. T.: Angiocardiographic findings in thoracoplasty, artificial pneumoperitoneum and phreniclasia. To be published.

[30] Yater, W. M., Finnegan, J., and Griffin, H. M.: Pulmonary arteriovenous fistula. J. A. M. A. **141:** 581, 1949.

ABSTRACTS

BACTERIAL ENDOCARDITIS

Lillehei, C. W., Bobb, J. R. R., and Visscher, M. B.: The Occurrence of Endocarditis with Valvular Deformities in Dogs with Arteriovenous Fistulas. Ann. Surg. **132:** 577 (Oct.), 1950.

The authors noted that the creation of large arteriovenous fistulas in dogs was associated with the subsequent appearance of endocarditis. It was not necessary to introduce any bacteria into the animals intentionally. Endocarditis occurred in 75 per cent of the dogs in which sufficiently large shunts existed for more than four weeks. Grossly, the endocardial lesions varied from soft friable vegetations to firm, smooth nodules; rupture of valve cusps was frequent. Vegetations in different animals were found to have affected the mitral, aortic, and tricuspid valves, the mural endocardium, and the site of the arteriovenous fistulas. Adrenal gland enlargement followed the creation of large arteriovenous shunts and preceded the development of valvulitis.

WESSLER

Boynton, R. D.: Subacute Bacterial Endocarditis Caused by Gaffkya Tetragena. New England J. Med. **243:** 738 (Nov.), 1950.

The author reports a case of subacute bacterial endocarditis caused by *Gaffkya tetragena* (*Micrococcus tetragena*) in a patient with chronic rheumatic valvular disease. This is the eleventh such case to appear in the world literature and the second in the American literature. The organism, generally considered as being of low virulence, nevertheless became rapidly resistant to streptomycin and penicillin, although the latter was given in massive dosage, and death occurred.

HANNO

McDonald, R. H.: Valvular Thrombotic Vegetation in Newborn ("Fetal endocarditis"). Arch. Path. **50:** 538 (Nov.), 1950.

The author reports an instance of fetal vegetative endocarditis on the tricuspid valve of a 17 hour old infant with a syphilitic mother who had received 4,800,000 units of penicillin in her fourth month of gestation.

The infant was premature, but appeared to be normal at birth. There was, however, a harsh systolic murmur over the precordium, a few rales were heard in the right lung, and the infant's blood Wassermann was positive. The actual cause of death is not clear. Autopsy revealed a normal heart except for a tricuspid vegetation measuring 8 by 4 mm., well attached by organization to the valve structure, in which there was fibroblastic activity, eosinophilic degeneration of the collagenous matrix and sparse polymorphonuclear infiltration. Bacteriologic study was negative, as was the search for spirochetes.

The author suggests that the vegetation was a bland thrombus formation in a ruptured valvular hematoma or blood cyst commonly seen along the free margins of the valvular leaflets in the newborn.

GOULEY

Highman, B., and Altland, P. D.: A New Method for the Production of Experimental Bacterial

Endocarditis. Proc. Soc. Exper. Biol. & Med. **75:** 573 (Nov.), 1950.

The authors state that bacterial endocarditis may be induced readily in rats exposed four hours daily to simulated high altitudes (25,000 feet). Twenty-six of 44 altitude rats and only one of 39 nonaltitude controls developed bacterial endocarditis after the intravenous injections of *Streptococcus mitis, Str. sanguis* and *Str. bovis* obtained from human cases of subacute bacterial endocarditis. Endocarditis was found in 20 of 26 altitude rats and in 7 of 14 nonaltitude controls injected with *Str. faecalis*. The incidence of endocarditis and the character of the lesions in other organs varied with the species of streptococcus used.

The authors suggested that altitude rats may be used to study the reaction and to test the effectiveness in vivo of various therapeutic agents against specific strains of organisms causing bacterial endocarditis in man. It was also suggested that exposure to altitude may be a useful experimental method for rendering resistant species of animals susceptible to some diseases other than endocarditis.

MINTZ

Villarreal, H., and Sokoloff, L.: The Occurrence of Renal Insufficiency in Subacute Bacterial Endocarditis. Am. J. M. Sc. **220:** 655 (Dec.), 1950.

The authors analyzed the records of 100 autopsied patients dying of subacute bacterial endocarditis. The available clinical data concerning renal function was correlated with the histopathologic lesions found in the kidneys of these patients. There were 14 instances of frank uremia found in the entire group and the probability of its existence in 9 other cases. Embolic glomerulonephritis was diagnosed as a pathologic entity in 9 patients. Acute, subacute, chronic or "combined" nephritis was found in the remainder of the 23 patients in whom renal insufficiency was found or suspected.

Blood cultures were obtained in 61 patients. Of 7 patients with renal insufficiency, only two had positive cultures ante mortem. *Streptococcus viridans* occurred in 30 of 47 patients without uremia. Although the patients with positive blood cultures did not frequently have renal lesions and the patients with renal lesions had few positive cultures, the authors conclude that subacute bacterial endocarditis resulting from *Str. viridans* may be second in importance only to beta hemolytic streptococcus as a cause of glomerulonephritis. The diagnosis of subacute bacterial endocarditis should be considered when renal insufficiency is found in a patient with valvular heart disease and fever.

SHUMAN

Zweifler, B., Sar, E., and Feder, I.: Subacute Bacterial Endocarditis Due to Streptococcus Fecalis. Ann. Int. Med. **34:** 217 (Jan.), 1951.

A 20 year old Negro female was admitted to the hospital because of fever, joint pains, and weakness. Examination revealed the presence of a migratory polyarthritis and a systolic apical cardiac murmur. The blood culture was positive for the presence of *Streptococcus faecalis*. Sensitivity tests indicated that the growth of the organism was inhibited by 16 units of streptomycin per cc. and by 16 units of penicillin per cc. Treatment was begun with streptomycin, a total of 138 Gm. being administered over a period of 29 days. In spite of this large dose, the blood cultures remained positive. For this reason, penicillin therapy was instituted, 350 million units over a period of 35 days being administered intramuscularly in conjunction with caronamide given orally. A cure followed the completion of this course of penicillin.

WENDKOS

BLOOD COAGULATION

Crosby, W. H., and Dameshek, W.: Paroxysmal Nocturnal Hemoglobinuria. The Mechanism of Hemolysis and Its Relation to the Coagulation Mechanism. Blood **5:** 822 (Sept.), 1950.

The authors noted that human serum became increasingly hemolytic against the red blood cells of patients with paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli Syndrome) during the course of several hours after the blood had been withdrawn and coagulated. This led them to an investigation of the possibility of a common factor being involved in coagulation and hemolysis.

A first series of experiments distinguished sharply between the paroxysmal nocturnal hemoglobinuria hemolytic system and the complement-antibody hemolytic system. All of the known factors in the normal coagulation mechanism were then investigated for their effect upon the paroxysmal nocturnal hemoglobinuria hemolytic mechanism. The hemolytic reaction was found to be an enzyme-like reaction and evidence was elicited suggesting that the heat-labile hemolytic factor in paroxysmal nocturnal hemoglobinuria, if not identical with the serum coagulation accelerator, resembles it in many respects, and is present in plasma as an inert precursor which is activated by thrombin.

Dicumarol was able to inhibit hemolysis in a patient with paroxysmal nocturnal hemoglobinuria but it did not reduce the hemolytic precursor and did not prevent hemolytic crises. However, it may be of value in the disease when thrombosis is a threat. Heparin, on the other hand, may be dangerous, since dilute heparin facilitates the activation of the hemolytic enzyme by thrombin.

It is suggested that some as yet undiscovered dicumarol-like drugs which would inhibit the production of serum Ac-globulin factor and of the hemolytic factor might offer a means of treatment for patients with paroxysmal nocturnal hemoglobinuria. In addition, a new approach to the coagulation problem is provided by the knowledge that paroxysmal nocturnal hemoglobinuria cells are lysed

by a substance related to the coagulation mechanism.

BEIZER

Stefanini, M., and Crosby, W. H.: Serum Prothrombin Time, A Composite Effect: An Analysis of the Factors Involved. Am. J. Clin. Path. **20:** 1026 (Nov.), 1950.

The one-stage prothrombin consumption test (serum prothrombin time) is theoretically dependent upon at least four factors: (1) the concentration of unconverted prothrombin in the serum; (2) the concentration of thrombin; (3) the "serum accelerator effect"; (4) and the amount of unconverted labile factor (factor V or plasma Ac-globulin).

The labile factor appears in excess and its concentration can be disregarded as a variable. In addition, if an adequate incubation period is allowed, thrombin is neutralized by the natural antithrombin. The serum prothrombin time, therefore, is influenced principally by the residual prothrombin concentration and the accelerator effect of serum.

The present studies demonstrate that in conditions characterized by limited thrombin formation and a high residual serum prothrombin activity (hemophilia, severe thrombocytopenia), the serum accelerator effect is weak. The authors conclude, therefore, that the serum prothrombin activity as determined by the one stage technic is a good index of the efficiency of the prothrombin conversion mechanism.

HANNO

Shapiro, S., Weiner, M., and Simson, G.: The Effect of Water-Soluble Preparations of Vitamin K in Dicumarol-Induced Hypoprothrombinemia. New England J. Med. **243:** 775 (Nov.), 1950.

This paper reports the effectiveness of large doses of water-soluble vitamin K preparations (Hykinone and Synkayvite), administered intravenously, in controlling excessive hypoprothrombinemia caused by dicumarol in 8 patients. Patients who were abnormally sensitive to the effects of dicumarol (that is, who show excessive prothrombin time prolongation with low plasma levels of dicumarol) responded satisfactorily within 24 hours after a single dose of water-soluble vitamin K, but repeated doses were necessary in those patients with hypoprothrombinemia who had high plasma dicumarol concentrations.

HANNO

Lawrence, J. S.: The Plasma Viscosity. J. Clin. Path. **3:** 333 (Nov.), 1950.

The author presents a method for estimating the plasma viscosity and the viscosities of the fibrinogen, globulin, and albumin fractions of plasma. Variations in the viscosities of the fractions reflect changes in the plasma proteins. Changes in the differential plasma viscosity pattern are largely nonspecific, occur in a wide variety of disorders and depend on the degree of acuteness or chronicity of the disease and the degree of involvement rather than on the nature of the morbid process itself.

HANNO

Goodwin, J. F., and Macgregor, A. G.: Anticoagulant Therapy with Heparin in Pitkin's Menstruum. Lancet **2:** 667 (Dec.), 1950.

The authors report that in 24 cases with thromboembolic phenomena, intramuscular heparin in Pitkin's menstruum was effective in all but three cases in doses of 200 to 400 mg. every 12 hours. There was a latent period of 6 to 48 hours before adequate clotting times were obtained necessitating the use of intravenous heparin where a rapid effect was needed. Coagulation times fluctuated considerably from day to day in the same patients and were markedly different from patient to patient as the result of differences in rate of absorption and sensitivity to heparin. They found the severity of local reactions and the tendency toward extensive bleeding into the injection site to be serious disadvantages.

BERNSTEIN

Muir, J. D.: Activity of Heparin in Pitkin's Menstruum. Lancet **2:** 671 (Dec.), 1950.

The author reports that in six normal subjects, heparin in Pitkin's menstruum, in 300 mg. dosage, gave effective prolongation of the clotting time from 9 to 12 hours in 5 cases and up to 21 hours in 1 case. Protamine sulfate in 1 per cent solution given intravenously was found to be effective in proper dosage in shortening the coagulation time. Ten cases with thromboembolic phenomena were treated with 8,000 to 10,000 I.U. intravenously and 300 to 400 mg. heparin/Pitkin subcutaneously with good results. No single dose was effective for more than 12 to 24 hours so dosage schedules were arranged on this basis depending on the clotting times.

BERNSTEIN

Applezweig, N., Walk, N., Vorzimer, J., and Sussman, L. N.: Studies on the Anticoagulant Action of Heparin. Am. J. Clin. Path. **20:** 1110 (Dec.), 1950.

The authors present a heparin assay technic based on the prothrombin time determination. Commercial preparations of heparin may be assayed rapidly by this method and the results closely approximate those obtained with the tentative U.S.P. method dependant upon per cent clot formation in recalcified plasma. Heparin in blood may be determined by the prothrombin time–heparin assay method if the accelerator factors are destroyed by boiling or ether extraction. The assay of heparin anticoagulant activity of blood, serum, plasma and unknown solutions has been made possible by this technic. With this method, the authors demonstrated the failure of blood to inactivate heparin even after 48 hours of incubation. They feel that

continued studies may provide further insight into the physiologic mechanisms controlling the in vivo action of heparin.

BEIZER

Sachs, J. J.: Experience with the Dilute Prothrombin Time in the Diagnosis of Thrombo-Embolic Disease, Am. J. M. Sc. **220:** 674 (Dec.), 1950.

In this study an evaluation was made of the acceleration of the dilute prothrombin time in relation to thromboembolic disease. In only one-third of the patients with intravascular clotting was the prothrombin time decreased to 28½ seconds or less. A similar number of control patients also manifested an accelerated prothrombin time. Therefore it was felt that this test did not aid in the diagnosis of thromboembolic disease.

It was demonstrated that patients with accelerated prothrombin times required no more dicumarol for maintanance of therapeutic levels than did patients with normal prothrombin times at the initiation of therapy. This evidence opposes the concept of hyperprothrombinemia in patients with accelerated prothrombin times. Fibrinogen determinations were performed simultaneously with the dilute prothrombin time in one group. Acceleration of the prothrombin time papeared to be associated with an elevated plasma fibrinogen level.

SHUMAN

CONGENITAL ANOMALIES

Gibson, S., Potts, W. J., and Langewisch, W. H.: Aortic-pulmonary Communication Due to Localized Congenital Defect of the Aortic Septum. Pediatrics **6:** 357 (Sept.), 1950.

The authors report findings in 4 patients strongly suggestive of a patent ductus arteriosus, who had an exploratory operation. In none of these cases was a patent ductus arteriosus found. All of the patients had a high pulse pressure, prominence in the region of the pulmonary conus, an atypical murmur, and a femoral thud. Three patients had systolic and diastolic murmurs at the base, increased prominence of vascular lung fields, and a capillary pulse. On the basis of these findings and the negative operative results, an aortic pulmonary communication due to a defect of the aortic septum was thought to be the most likely diagnosis. There was no postmortem evidence because all of the patients recovered postoperatively.

Convincing evidence in the first case was that cardiac catheterization failed to demonstrate an intracardiac shunt; there was a markedly increased pulmonary blood flow; and at operation the thrill was completely eliminated by pressure near the origin of the aorta and pulmonary artery. In the second case, catheterization studies were compatible with an aortic-pulmonary communication; the angiocardiogram showed a second filling of the lung coincident with filling of the aorta; and at operation, a definite thrill was palpated over the origin of the pulmonary artery. In the last 2 cases at operation a thrill was found in the same location.

Sixteen cases have been previously reported in the literature. The defect is fairly uniform in size and location, varying from 0.5 cm. to 1.5 cm. in diameter, and is situated a short distance above the semilunar valves. The authors believe that the lesion may be more frequent than the published reports indicate. They further suggest the possible use of retrograde aortography to establish the diagnosis and thus spare an exploratory operation.

MARGOLIES

Kjellberg, S. R., and Rudhe, U.: Electrokymographical Studies of Coarctation of the Aorta. Acta radiol. **34:** 145 (Sept.), 1950.

The authors describe the electrokymogram of 12 patients with coarctation of the aorta. The electrokymographic curve distal to the stenosis differs from that in the ascending aorta in that the onset is later, the rise occurs more slowly and the notches on the descending limb tend to disappear. These effects are similar to the relationship of the pulse waves in the femoral artery as compared to those in the carotid artery.

SCHWEDEL

Sweeney, D. B., and Patton, W. B.: Surgical Management of Cerebral Abscess Associated with Congenital Heart Disease. South. M. J. **43:** 799 (Sept.), 1950.

Cerebral abscess occasionally complicates congenital heart disease. Surgical "cures" have rarely been reported. This paper describes two instances with good therapeutic results. A 10 year old boy with probable Eisenmenger complex had an abscess in the left posterior parietal region. Aspiration and penicillin led to considerable improvement although a residual slight hemiparesis remained. In an 8 year old boy with the tetralogy of Fallot a subcortical abscess developed three years after a Blalock operation. Surgical and antibiotic therapy led to prompt recovery. No organism was isolated in either case. Since the cerebral abscess is usually single it is potentially amenable to surgery and the prognosis is improved by modern antibiotics.

WAIFE

Maronde, R. F.: Brain Abscess and Congenital Heart Disease. Ann. Int. Med. **33:** 602 (Sept.), 1950.

The authors report 81 cases of congenital heart disease in 13,883 autopsies. Of this group there were 5 examples of the tetralogy of Fallot; 3 examples of associated ventricular and atrial septal defects; 2 of ventricular and atrial septal defects associated with a rudimentary right ventricle; 11 of an isolated ventricular septal defect. Cerebral abscesses were observed in 4 of the cases of the tetralogy of Fallot: in 2 of the cases of associated ventricular and atrial

septal defects; in 1 of the cases with a rudimentary right ventricle associated with ventricular and atrial septal defects; in 4 of the cases with an isolated ventricular septal defect. It is believed that the shunting of blood into the systemic arterial vessels without the benefit of the filtering action of the pulmonary capillaries plays an important role in the etiology of these abscesses.

WENDKOS

Ravin, A., and Darley, W.: Apical Diastolic Murmurs in Patent Ductus Arteriosus. Ann. Int. Med. **33:** 903 (Oct.), 1950.

The author found that in 9 of 21 patients a patent dustus arteriosus was demonstrable and an apical diastolic murmur was heard. In 5 of this number, phonocardiographic studies confirmed the presence of an apical diastolic murmur which could readily be confused with the murmur of mitral stenosis. The apical diastolic murmur associated with a patent ductus arteriosus is ascribed to (a) enlargement of the left ventricular cavity secondary to the increased work imposed upon the left ventricle by reason of the shunt, (b) increased rate of blood flow through the mitral valve, (c) increased blood volume reaching the left auricle. The enlarged left ventricular cavity distal to a normal mitral ring creates the situation of a relative mitral stenosis, and sets the stage for the production of a diastolic murmur when the other factors already mentioned are also operating.

WENDKOS

CONGESTIVE HEART FAILURE

Lawne, J. A., Schemm, F. R., and Hurst, W. W.: Further Comparative Studies on Ascites in Liver and Heart Disease. Gastroenterology **16:** 91 (Sept.), 1950.

Comparative studies were carried out in patients who had gross hepatomegaly and ascites as a result of congestive heart failure and in cases of portal cirrhosis with ascites. In both groups the sodium and chloride excretion in the urine was found to follow a similar pattern during the period of clearing of ascites and edema. The cardiac patients showed almost consistently abnormal bromsulfalein dye retention values when freed of their ascites and edema. The bilirubin and total protein content of subcutaneous tissue fluid and of pleural and peritoneal fluid was less than that of plasma. However, there was no constant relationship between the ratio of plasma bilirubin over plasma protein to the ratio of subcutaneous tissue fluid bilirubin over tissue fluid protein, or to the ratio of pleural or abdominal fluid bilirubin over protein in these fluids.

The authors concluded that a permanent impairment of hepatic function may exist in the cardiac patients studied and that it may add to the effects of cardiac decompensation in disturbing water and sodium balances.

SCHWARTZ

White, A. G., Gordon, H., and Leiter, L.: Studies in Edema. II. The Effect of Congestive Heart Failure on Saliva Electrolyte Concentrations. J Clin. Investigation **29:** 1445 (Nov.), 1950.

The concentrations of electrolytes in the saliva of patients with congestive heart failure on a low-salt regimen and on a regular salt intake were compared to control noncardiac subjects. It was found that congestive failure was associated with lowered sodium and chloride and higher potassium concentrations in the saliva, when compared with normal subjects. However, those on a salt-poor diet did not show significant differences in the electrolyte concentrations of saliva. No relationship was found between serum electrolyte and saliva electrolyte concentrations.

WAIFE

Noehren, T. H., and McKee, F. W.: Cardiac Failure in a "Normal" Heart. Ann. Int. Med. **33:** 1485 (Dec.), 1950.

The authors report the case of an extremely obese 53 year old white male who developed auricular fibrillation and left ventricular failure, from which he succumbed eight days later, after undergoing a combined abdominoperineal resection of a carcinomatous bowel. Except for the finding of some fatty infiltration of the heart, the necropsy did not reveal any significant cardiac pathology. The myocardial fibrils were normal and the coronary vessels were normal except for slight intimal thickening. The endocardium was thin and a few areas of fat deposition were noted around the subendocardial vessels. It is suggested that this case may be an example of cardiac failure induced by physiologic disturbances within the myocardium, inasmuch as significant structural cardiac changes could not be demonstrated following microscopic examination of the cardiac tissues.

WENDKOS

Proger, S. and O'Connor, J. J.: Intractable Heart Failure. Ann. Int. Med., **33:** 1349 (Dec.), 1950.

In this discussion, the authors have attempted to indicate some of the problems involved in the therapy of intractable heart failure and some of the immediate areas of attack. Particular emphasis is placed upon the electrolyte disturbances which can accrue in cases of heart failure with or without an intensive diuretic program. The proper identification of the disturbed relationships of the various constituents within the electrolyte partition of the body assumes importance in such cases because the reversibility of heart failure is largely dependent on their correction. For this reason, free use should be made of modern laboratory technics for the detection of hyponatremia, hypochloremia, acidosis, alkalosis, hyperkalemia, hypokalemia and azotemia. The authors feel it is important to recognize the prerenal factors which constitute additive causes of the pronounced renal insufficiency often seen in intractable

heart failure, because these factors are reversible. Unquestionably, the chemical disturbances associated with heart failure represent currently an area for therapeutic exploration which will be pierced and exploited as investigators attain a clearer view of the biochemical problems involved.

WENDKOS

Haft, H. H., and Adler, D. K.: Thoracic Stomach Simulating Left Ventricular Failure. Ann. Int. Med. **33:** 1472 (Dec.), 1950.

The authors report the case of a 71 year old female who had experienced for two years sudden episodes of dyspnea associated with a sensation of heaviness in the chest. The attacks usually persisted for an hour or two, were usually provoked by the ingestion of a heavy meal and were relieved by assuming the prone position. Aside from a moderate degree of obesity, physical examination did not disclose any significant findings. X-ray study of the esophagus and stomach established a dome-like shadow in the chest to be an air filled thoracic stomach lying in the posterior mediastinum. The pylorus occupied a position just below the right leaf of the diaphragm. The electrocardiogram was normal. It is considered that interference with the venous return of blood by the obstruction produced by the distended thoracic stomach might have been an important factor contributing to the paroxysmal dyspnea simulating left ventricular failure, although there is the possibility that reflex diminution of coronary flow induced by the distended viscus in the thorax might have been responsible for the symptoms.

WENDKOS

CORONARY ARTERY DISEASE, MYOCARDIAL INFARCTION

Laplane, R., and Pautrat, J.: Experimental Myocardial Infarction by Subadventitial Irritation of the Aorta. Arch. d. mal. du coeur **43:** 888 (Oct.), 50.

The authors found that injection of an irritant (croton oil, privine or cantharidine) beneath the adventitia of the ascending aorta in rabbits and dogs was followed by th' development of severe myocardial lesions and by significant changes of the electrocardiogram. The histologic changes of the myocardium consisted in vascular lesions, hemorrhagic interstitial infiltration and degenerative alterations of the muscle fibers reaching the state of colliquative necrosis, and were usually scattered throughout the myocardium. In one experiment, a confluent necrosis of the anterior wall was seen macroscopically. The electrocardiographic changes consisted in T wave alterations, in QRS and T anomalies of the type seen in myocardial infarction, and in disturbances of the cardiac rhythm (bradycardia and premature beats).

The authors consider the possibility of a reflex mechanism in the development of myocardial infarction, which could account for myocardial necrosis observed in the presence of normal coronary arteries, in pulmonary embolization or in disease of the aortic wall.

PICK

Vineburg, S. M., and Niloff, P. H.: The Value of Surgical Treatment of Coronary Artery Occlusion by Implantation of the Internal Mammary Artery into the Ventricular Myocardium; an Experimental Study. Surg., Gynec. & Obst. **91:** 551 (Nov.), 1950.

In the past there have been four main approaches in the attempt to augment the circulation of the ventricular myocardium in cases of coronary artery sclerosis: (a) the application of a graft to the surface of the heart; (b) cardiopericardiopexy; (c) cardiac vein ligation; (d) arterialization of the coronary venous system. Further studies of vascularization of the ventricular myocardium by left internal mammary artery implantation are reported. Macroscopic and microscopic findings of animals developing collateral circulation are shown and described. The frequency of anastomotic formation and the degree of protection afforded by these anastomoses are discussed. An anastomosis has been shown to occur between the internal mammary artery and the left ventricular circulation in from 50 to 73 per cent of the animals. The degree of protection against coronary occlusion varies according to the size of the anastomotic channels. When there is a large anastomosis present, death and infarction from coronary occlusion do not occur. Thrombosis of the implant limits the size of the anastomosis. When thrombosis occurred, in many instances small anastomoses developed through recanalized channels. The operation is technically simple and appears not to damage the heart. Several advantages of the procedure and its potential value in certain human cases of coronary insufficiency are emphasized.

BECK

Chitwood, W. R.: The Importance of Recognizing Post-Infarctional Shoulder-Hand Syndrome. New England J. Med. **243:** 813 (Nov.), 1950.

The authors, with the aid of two illustrative case reports, stress the importance of recognizing the painful shoulder-hand syndrome which often follows myocardial infarction. A correct diagnosis will prevent needlessly prolonged bed rest, occasioned by the mistaken impression that the pain in patients recovering from a myocardial infarction represents continued myocardial damage; and inappropriate therapy based upon erroneous diagnoses of arthritis, bursitis, psychoneurosis, and other conditions may be avoided.

HANNO

Bresnick, E., Selverstone, L. A., Rapoport, B., Chesky, K., Hultgren, H. N., and Sise, H. S.: Experiences with Dicumarol in Acute Myocardial

Infarction. New England J. Med. **243:** 806 (Nov.), 1950.

The authors present a study of the effects of dicumarol in a series of 122 cases of acute myocardial infarction, as compared with the results in 128 control cases treated without anticoagulants. The incidence of peripheral arterial emboli and phlebothrombosis was lower in the treated group (26 thromboembolic episodes in 19 cases) than in the controls (32 thromboembolic episodes in 26 cases), but the total mortality figures were higher (18.9 per cent and 12.5 per cent, respectively, in the treated and untreated cases). Bleeding complicated anticoagulant therapy in 10 cases, with two deaths resulting from hemorrhage.

The authors conclude that Dicumarol, as administered under routine hospital conditions, is largely ineffective in the treatment of acute myocardial infarction. Variations in the sensitivity of different patients to Dicumarol, inexperience on the part of the different physicians handling the cases in maintaining safe but therapeutically effective prothrombin levels, and the unavailability of prothrombin time determinations on weekends and holidays, all militate against ideal results with dicumarol.

HANNO

Harris, A. S., and Kokernot, R. H.: Effects of Diphenylhydantoin Sodium and Phenobarbital Sodium Upon Ectopic Ventricular Tachycardia in Acute Myocardial Infarction. Am. J. Physiol. **163:** 505 (Dec.), 1950.

The authors feel that apparent similarities between origins of delayed ectopic discharges following coronary occlusion and focal epileptogenic discharges suggests that such drugs as Dilantin and phenobarbital sodium might suppress ectopic ventricular discharges which accompany acute myocardial infarction. The anterior descending branch of the left coronary was occluded in two stages, 30 minutes apart. No animals were lost by fibrillation by this method. After 4.5 to 8 hours ectopic ventricular tachycardia develops. Ectopic frequencies above 180 per minute require 125 to 200 mg. per Kg. of Dilantin sodium intravenously in divided doses to reduce the rate to zero and keep it at less than one half control rates for five hours. At rates below 170 Dilantin in total amounts of 100 mg. per Kg. or less is effective.

Phenobarbital sodium alone reduced ectopic rates briefly. When two doses of Dilantin sodium (total 25 mg. per Kg.) followed, all ectopic beats were eliminated for two hours and the ectopic rate had only returned 30 per cent in six hours. It is concluded that prior and intercurrent use of phenobarbital increases the effectiveness of Dilantin and reduces the amount required to suppress ectopic activity. Dilantin also depresses the pacemaker, but to a lesser extent than ectopic foci. Dilantin sodium must be given slowly. Rapid injections produce an acute hypotension and hyperpnea. In a few trials the administration of sufficient Dilantin alone to suppress all ectopic activity produced restlessness, rigidity of limbs, and opisthotonous. After the use of phenobarbital, the relatively small amounts of Dilantin needed did not cause such symptoms.

OPPENHEIMER

Evans, J. A., Poppen, J. L., and Tobias, J. B.: Relief of Angina Pectoris by Sympathectomy. J. A. M. A. **144:** 1432 (Dec.), 1950.

The present study is a report on resection of the sympathetic anginal pathway including the first to the fourth thoracic ganglions on both sides. Ten patients who were subjected to this procedure had satisfactory relief. Three patients had complete relief of pain, but there was a residual sense of throat constriction. The authors feel that the pernicious cycle of anginal pain in status anginosis can be broken by sympathectomy on the same basis that reflex sympathetic dystrophy is helped. They do not feel that Horner's syndrome is a valid contraindication to carrying the resection to the lower stellate ganglion, especially when the resection is bilateral. Findings suggest that good results might be expected in cases of the shoulder hand syndrome and Dupuytren's contracture. Disadvantages of the operation include ptosis or Horner's syndrome, postural hypotension, and severe sweating in the groins with swollen nasal membranes. The last symptom tends to subside early.

KITCHELL

ELECTROCARDIOGRAPHY

Franke, H., and Gebert, E.: Asynchronous Excitation of the Auricles in the Intracardiac Electrocardiogram of the Healthy Organism. Ztscht. f. Kreislaufforsch. **39:** 513 (Sept.), 1950.

The authors studied the spread of impulse in the auricles in 4 normal dogs and one monkey by variously located leads from the cavity of the right auricle taken simultaneously with esophageal leads. From a circumscribed area in the right auricle, located between the orifice of the superior vena cava and the auricular septum, bifid P waves were regularly obtained. The first peak of this wave corresponded to the peak of P, obtained in other parts of the right auricle, while the second peak occurred simultaneously with the auricular deflection of the esophagogram. Histologic examination of the area, which yielded biphasic P waves, revealed no pathologic lesion but the presence of muscle fibers resembling those of the specific conduction system. The authors conclude that the impulse transmission from the right to the left auricle is confined to a specific conduction system, which was as described previously in a similar location as Bachmann's interauricular bundle.

PICK

Scherlis, L., Sandberg, A. A., Wener, J., Dvorkin, J., and Master, A. M.: The Effects of the Single and Double "Two-Step" Exercise Tests upon the Electrocardiogram of 200 Normal Persons. J. Mt. Sinai Hosp. **17:** 242 (Nov.–Dec.), 1950.

The authors studied two hundred normal males and females varying in age from 17 to 57 years who were free of cardiac complaints and had a normal physical examination. Following the recording of the normal electrocardiogram, the subjects were given the single two step test, and standard leads I, II, and III and V_4 or V_5 were taken immediately and one, two, and five minutes afterward. At least 24 hours later, the procedure was repeated. The electrocardiograms were analyzed for rate, RS-T segment deviation, P and T wave alterations, QRS complexes, and arrhythmias.

On the basis of the criteria pertaining to the RS-T segment depression, 2.5 per cent of the 200 normal persons had positive single two step tests, 5.5 per cent had positive double two step tests, and 0.5 per cent had either positive single or positive double two step tests or both. In four persons without associated RS-T segment depression, the T waves became flat immediately after exercise. There was no instance of inversion of the T wave in lead I, II, or V_4. Increased amplitude of the P wave occurred in a few instances. The P-R interval was essentially unaltered. The authors discuss the advantage of the precordial lead in detecting RS-T segment changes, and particularly the advantage of the V lead over the CF lead, because in the latter lead any negativity recorded in the precordial lead would be offset by negativity recorded in the left leg.

CORTELL

Pastor, B. H., and Worrilow, S. H.: Electrocardiographic Patterns in Stokes-Adams Syndrome. Ann. Int. Med. **34:** 80 (Jan.), 1951.

An 82 year old male died during a syncopal attack which lasted three hours and during which repeated convulsio ns occurred. In this terminal unconscious state, a series of electrocardiograms was taken. At the beginning, the standard limb leads showed complete A-V heart block with an idioventricular rate of 30 per minute, and coupled ventricular extrasystoles. Later he developed a paroxysm of prefibrillary ventricular tachycardia (ventricular flutter) with a rate of 300 per minute, during which there were a number of cycles of ventricular fibrillation, followed by a period of ventricular asystole during which the auricles continued to beat at a rate of 70 per minute. Finally, a prolonged period of ventricular tachycardia occurred which continued for a period of about 10 minutes. Abrupt cessation of this mechanism with complete standstill of the galvanometer string occurred at a moment when the machine had been stopped for rewinding, and only a feeble ventricular response could be produced with the intracardiac injection of adrenaline. Necropsy revealed moderate dilatation of the heart but no definite cardiac enlargement. There were small areas of fibrosis, but no gross evidence of any acute myocardial process was visible. Except for sclerotic changes at the base of the aortic valve and septum, there were no findings of significance in the rest of the examination, including the microscopic study. This case illustrates the importance of recognizing the underlying cardiac mechanism in Stokes-Adams syncope. The treatment will differ significantly depending upon whether the syncope is due to a heterotopic ventricular rhythm or to ventricular standstill.

WENDKOS

Furman, R. A., Hellerstein, H. K., and Startzman, V. V.: Electrocardiographic Changes Occurring During the Course of Replacement Transfusions. J. Pediat. **38:** 45 (Jan.), 1951.

Serial electrocardiograms were taken on 6 patients ranging in age from 4 to 26 hours old, during the exsanguination replacement procedure. Four hundred eighty to 580 cc. of citrated blood was administered over 70 to 130 minute periods. Ten cc. of 10 per cent calcium gluconate was given intravenously over a two to four minute period at the conclusion of the transfusion.

There were electrocardiographic changes characteristic of hypocalcemia during the replacement procedure. In 4 patients there were marked fine skeletal muscle tremors which obscured the P and T waves, thus preventing the measurement of electrical systole. However, in the other 2 patients there was an increase in the Q-T interval from 0.422 and 0.428 to 0.525 and 0.512 seconds respectively. The Q-T interval increase was due to a prolongation of the S-T segment. A slight decrease in the amplitude of the T waves in leads I and aV_L also appeared. There was no change in the QRS complex, the duration of the P-R interval, or the electrical position. In 2 patients, the heart rate increased 20 or more beats per minute.

The intravenous administration of calcium reversed the electrocardiographic picture abolishing the muscle tremors and returning the Q-T interval to normal. However, changes characteristic of hypercalcemia appeared after all the calcium had been absorbed. There was a constant reduction in the heart rate from 24 to 34 beats per minute, and a constant decrease in the Q-T interval to below the value at the start of the procedure. In 4 cases the T waves became isoelectric or inverted immediately after the calcium had been given. During the course of the transfusions, 2 patients showed slight elevations and 2 showed slight reductions in total serum calcium. This demonstrates that in the presence of a normal or elevated serum calcium, the electrocardiogram can detect a reduction in ionized calcium.

MARGOLIES

Segers, M.: Determinism of Preponderance Curves. Acta cardiol. **5**: 288, 1950.

After studying various degrees of ventricular aberration following conduction of auricular premature beats, the author presents his concept of ventricular preponderance as an expression of a conduction delay within the septum. In contrast to patterns representing bundle branch block and conduction defects in the free wall (peripheral block), preponderance patterns are not associated with a delay of the intrinsicoid deflection in either left- or right-sided chest leads. However, septal conduction delay (preponderance) can be associated with a peripheral block and may produce unusual patterns. Right axis deviation, sometimes seen in disease of the left heart, and the "concordant" type of left heart strain can, in the author's opinion, be explained by the combination of a delay in activation of the left side of the septum and of the free wall of the right ventricle.

PICK

Donzelot, E., Durand, M., and Metianu, Z.: Some Considerations on Congenital Auriculoventricular Dissociation and Presentation of Two Personal Observations. Acta cardiol. **5**: 319, 1950.

A-V block of congenital origin is a rare disturbance; only about 30 true examples have been presented in the literature. The authors report 2 of their own observations among 650 cases of congenital heart disease, in which an electrocardiogram was available. The first was an infant with situs inversus, in whom the disturbance of the heart rhythm was detected at the age of 4 months. In the second patient, with signs of Eisenmenger's disease, the congenital origin of the heart block was uncertain, since the presence of heart block was first found at the age of 7 years.

PICK

ENDOCRINE EFFECTS ON CIRCULATION

Myers, J. D., Brannon, E. S., and Holland, D. C.: A Correlative Study of the Cardiac Output and the Hepatic Circulation in Hyperthyroidism. J. Clin. Investigation **29**: 1069 (Aug.), 1950.

The authors studied the hepatic blood flow and cardiac output by the catheterization technic in 40 patients with active hyperthyroidism. In spite of a definitely increased cardiac output the hepatic blood flow was not significantly increased. The splanchnic blood flow was essentially normal but there was increased oxygen extraction in hyperthyroidism. Regional oxygen consumption was greater than the over-all metabolic rate in this disease, and may partially explain the centrolobular anoxia and necrosis.

There was an elevation of systolic and mean pressure, but not of diastolic pressure, in the right ventricle and pulmonary artery. Associated with this there was an elevated pulmonary blood flow but a normal pulmonary peripheral vascular resistance.

This data indicates that normal peripheral vascular resistances are found in the lungs, splanchnic area, and brain, in the presence of an over-all decrease in resistance. This suggests that a reduction in resistance occurs in such sites as the skin.

WAIFE

Scheinberg, P., Stead, E. A., Jr., Brannon, E. S., and Warren, J. V.: Correlative Observations on Cerebral Metabolism and Cardiac Output in Myxedema. J. Clin. Investigation. **29**: 1139 (Sept.), 1950.

Using the nitrous oxide method, cerebral blood flow and metabolism was measured in 8 patients with myxedema. In 7 other myxedematous subjects the cardiac output was determined by the direct Fick method.

It was found that the cardiac output was reduced (47 per cent from normal) and that the total vascular resistance of the body was increased in myxedema.

Cerebral blood flow was reduced (38 per cent), as was the cerebral oxygen consumption (27 per cent), although cerebral vascular resistance was increased greatly (91 per cent). An excellent correlation was found between the basal metabolic rate and the cerebral blood flow.

Three patients restudied after thyroid therapy had a definite return toward normal in their cerebral metabolic functions.

It is suggested that the mental changes in myxedema are the result of decreased cerebral oxygen and glucose metabolism and that the reduction in cardiac output is secondary to a generalized reduction in organ blood flow.

WAIFE

Friedman, S. M., Friedman, C. L., and Nakashima, M.: Action of Cortisone on Cardiovascular-Renal Effects of Desoxycorticosterone Acetate. Am. J. Physiol. **163**: 319 (Nov.), 1950.

The rise in blood pressure which ordinarily follows administration of DCA did not occur in the presence of cortisone. Increased heart size and presence of renal damage in DCA-cortisone treated animals are taken to indicate that cortisone cannot be considered antagonistic to the cardiovascular-renal effects on DCA. The animals in these experiments did not gain weight. This fact, coupled with a reduction in the number and quality of pituitary eosinophiles, suggests a possible mechanism to account for the inhibition of growth. The effect of cortisone on blood pressure may be due to failure to gain weight. The ability of Cortisone by itself to elevate plasma potassium and chloride is more than overbalanced when DCA is given simultaneously. Cortisone, in the doses used (2 mg. daily), produced histologic

renal glomerular lesions which were added to those of DCA when the two were given simultaneously.

OPPENHEIMER

Dieckmann, W. J., Egenolf, G. F., Morley, B., and Pottinger, R. E.: The Inactivation of the Antidiuretic Hormone of the Posterior Pituitary Gland by Blood from Pregnant Patients. Am. J. Obst. & Gynec. **60:** 1043 (Nov.), 1950.

The authors incubated Pitressin with citrated blood of patients in the latter half of pregnancy. When this material was injected into normal subjects undergoing water diuresis there was little or no decrease in the urinary output. However, when Pitressin and blood from nonpregnant patients was given, antidiuresis developed.

In the nonpregnant controls, the concentration of chlorides in the 90 minute volume was markedly increased, whereas the chlorides in the pregnant blood experiments were only slightly increased or even lowered. Sodium and potassium levels in the urine paralleled the chloride results. The authors conclude that blood in the latter half of pregnancy has the ability to inactivate the antidiuretic affect of commercial Pitressin.

WAIFE

Kyle, L. H., and Knop, C. Q.: Simulation of Cardiac Disease by Adrenocortical Failure in Infants. New England J. Med. **243:** 681 (Nov.), 1950.

Congenital adrenocortical hyperplasia is a familial disorder, characterized by increased production of adrenal androgen and decreased production of the electrolyte regulating hormones. Although newborn females with this disease invariably have obvious genital abnormalities, male infants ordinarily demonstrate no gross disorder of sexual development. The characteristic increase in 17-ketosteroid excretion is essential for diagnosis in the male. Adrenal insufficiency with hyperpotassemia is a common complication.

The authors report 3 such cases in siblings, in 2 of which marked abnormality in cardiac rhythm was a dominant clinical feature. Electrocardiographic studies on one child revealed a normal sinus rhythm with left bundle-branch block and partial auriculoventricular block, followed some hours later by numerous ventricular beats occurring frequently in pairs. An electrocardiogram in the second case disclosed auricular fibrillation with periods of complete heart block, and a later tracing showed a normal sinus rhythm with definite hyperpotassemic effects. The authors recommend the V leads for detecting hyperpotassemic changes.

HANNO

Bell, G. O.: Hyperthyroidism, Pregnancy and Thiouracil Drugs. J. A. M. A. **144:** 1243 (Dec.), 1950.

Twenty-one patient are presented with the study of three factors: the mother and the hyperthyroidism, the pregnancy and its complications, and the child. The author concludes that subtotal thyroidectomy after proper preparation can be done during the first two trimesters. Full term living infants born to such patients have been normal. In 15 cases followed to term there were ten full term normal deliveries with eleven healthy children; one abortion at five months; two premature births at eight months; one case of toxemia at term with death of the infant and one caesarian section with death of the infant. This represents a fetal loss of 33 per cent. Discussions are presented which show dangers of hypothyroidism subsequent to thyroidectomy or induced by antithyroid drugs. Therapy directed towards fetal salvage is suggested including avoidance of overdosage with antithyroid drugs, administration of thyroid and iodine, and possible use of diethylstilbestrol during pregnancy.

KITCHELL

Goldstein, M. S., Ramey, E. R., and Levine, R.: Relation of Muscular Fatigue in the Adrenalectomized Dog to Inadequate Circulatory Adjustment. Am. J. Physiol. **163:** 561 (Dec.), 1950.

The authors measured work performance of gastrocnemius muscle and arterial blood pressure simultaneously in 2 normal and 4 desoxycorticosterone-treated adrenalectomized dogs. The adrenalectomized dogs had 605 to 650 mg. per cent of NaCl and glucose 60 to 87 mg. per cent. Three of the adrenalectomized dogs had blood pressures of 95, 110, 150 mm. Hg, the fourth was 70 mm. Hg. Normal dogs survived direct muscle stimuli at rates of 3 per second for 6 and 12 hours without fall in blood pressure or decrease in muscle contractions. In the three adrenalectomized animals blood pressure, after being sustained for a time, fell to levels of 60 to 75 mm., at which levels decreased muscle contractions appeared 10 to 45 minutes after onset of falling blood pressures. Blood pressure fell until the dogs died. The dog with the low pressure had a rapid fall in blood pressure and an immediate decrease in contraction of skeletal muscle. Death occurred in 60 minutes. A relation between blood supply and fatigability in the intact adrenalectomized animal is demonstrated. Transfusion or pressor drugs produce temporary elevation in blood pressure and partial or complete restoration of the initial height of contraction. It is emphasized that blood pressure fall preceded signs of muscular fatigue. It is further suggested that failing work performance of adrenalectomized animals is a result of circulatory maladjustment to demands of work. The role of C-11 oxysteroid lack in relation to fatigue is stressed.

OPPENHEIMER

HYPERTENSION

Kreuziger, H., and Dreiheller, H.: The Question of Determination of Cholinesterase in the Blood

Serum of Hypertensive Patients. Ärztl. Wchnschr. **5**: 675 (Sept.), 1950.

The authors determined the level of cholinesterase in the blood serum of 40 normotensive persons and in 50 hypertensives of various etiology. Differences in the level of cholinesterase found in the two groups were not of statistical significance. The cholinesterase content of the serum does not permit a distinction of various forms of hypertension, nor can it be correlated with the degree of pressure elevation.

PICK

Wunsch, R. E., Warnke, R. D., and Myers, G. B.: The Effects of Dibenamine on Severe Hypertension. Ann. Int. Med. **33**: 613 (Sept.), 1950.

The authors treated 14 patients with severe hypertenion with a total of 39 infusions of a dilute mixture of Dibenamine. In all patients, the first effect was pupillary constriction. The effect on the blood pressure was variable in degree and duration, and could not be correlated directly with either the dosage or rate of injection. The most striking therapeutic effect onsisted in the relief, for from three days to several weeks, of hypertensive headache in seven patients in whom headache had been refractory to all other measures. Distinct improvement in vision was also noted in three patients. Three patients who were very irritable before receiving Dibenamine became docile and cooperative after administration of the drug. Toxic reactions consisted of nausea, vomiting, drowsiness, restlessness, mental confusion and convulsions. By appropriate tests, visceral damage by this drug could not be demonstrated. Seventeen ambulatory patients with a benign type of essential hypertension received the drug by the oral route, but all developed nausea and vomiting following ingestion of amounts which were effective in either lowering the blood pressure or lessening the response to the cold pressor test. It is concluded that Dibenamine is clinically useful only by the intravenous route in the hospitalized patient with severe hypertension and hypertensive encephalopathy. Intravenous Dibenamine is contraindicated in the treatment of the ambulatory hypertensive patient not only because of its toxicity, but also because of the profound and protracted orthostatic hypotension which results from its administration by this route.

WENDKOS

Olsen, N. S., and Schroeder, H. A.: Oxygen Tension and pH of Renal Cortex in Acute Ischemia and Chronic Hypertension. Am. J. Physiol. **163**: 181 (Oct.), 1950.

In dogs under Nembutal both unilateral acute renal ischemia and unilateral chronic renal arterial constriction associated with elevation of blood pressure were accompanied by a consistent alteration of pH of the cortex of the kidney toward the acid side as compared with the normal kidney. Under the same circumstances oxygen tension was also lowered. Acidification of the cortex did not depend entirely on hypoxia. After moderate degrees of clamping of the renal artery oxygen tensions fell and then readjusted to control values. The decided change in pH toward acidity persisted under these conditions. As a result, slight renal ischemia with a normal oxygen tension and an acid pH obtained. Both acute and chronic unilateral preparations were less sensitive to small doses of epinephrine than the control normal kidney.

OPPENHEIMER

Watkin, D. M., Froeb, H. F., Hatch, F. T., and Gutman, A. B.: Effects of Diet in Essential Hypertention. I. and II. Am. J. Med. **9**: 428, 441 (Oct.), 1950.

The first paper, *Baseline Study: Effects in Eighty-Six Cases of Prolonged Hospitalization on Regular Hospital Diet* presents complete baseline studies in 86 hospitalized patients with blood pressures of 220/120 or more who ate regular hospital diet for a mean period of 9 weeks. Twenty to 50 per cent of the patients improved symptomatically, especially those with cardiac failure. Headaches persisted, however, and the blood pressure changed little. The variations in response to hospitalization were so great and unpredictable that each subject had to serve as his own control.

The second paper, *Results with Unmodified Kempner Rice Diet in Fifty Hospitalized Patients*, presents data on 50 patients with essential hypertension who were maintained on the Kempner rice diet under controlled hospital conditions for a mean period of 10½ weeks after a control period of 10 weeks. The results were in essential agreement with those described by Kempner. Nine patients failed to improve, but marked symptomatic improvement occurred in many patients. The most serious complication of the rice diet was the activation of obsolescent peptic ulcers.

On the rice diet, a mean fall of 29 mm. Hg and 16 mm. Hg respectively in the systolic and diastolic pressures occurred. The fall in pressure was related largely to the low salt content of the diet. Cardiac catheterization showed a lowered peripheral vascular resistance. No significant change occurred in the mean serum concentration of sodium, calcium, and inorganic phosphate; the mean serum potassium and bicarbonate rose; and the serum chloride fell significantly. Liver impairment was suggested by the decline in the cholesterol esters, the rise in serum neutral fats, and the changes in the liver function tests.

The authors believe the rice regime, because of its low sodium content, specifically reduces the blood pressure in a significant proportion of patients with severe essential hypertension. Cholesterol determinations before and during treatment are recommended to appraise the effects of the rice diet. Protracted effective maintenance of the Kempner regime imposes such hardship upon the patient as

to render it virtually impracticable for general use. Modifications of the diet are suggested.

HARRIS

Starke, H.: Effect of the Rice Diet on the Serum Cholesterol Fractions of 154 Patients with Hypertensive Vascular Disease. Am. J. Med. **9:** 494 (Oct.), 1950.

The rice diet produced a significant fall in the total, free, and esterified serum cholesterol levels in a large group of patients with hypertensive vascular disease. Prior to the beginning of the rice diet 29 of 154 patients had a total serum cholesterol of 220 mg. per 100 cc. or less; 124 patients had a total serum cholesterol concentration greater than 220 mg. per 100 cc. The mean ratio of free to total cholesterol in the first group remained unchanged during the diet. In the second group of patients whose initial total serum cholesterol was 221 mg. per 100 cc. or more, the mean ratio of free to total serum cholesterol increased during the rice diet.

HARRIS

Smith, D. E., Odel, H. M., and Kernohan, J. W.: Causes of Death in Hypertension. Am. J. Med. **9:** 516 (Oct.), 1950.

Analysis of the causes of death in 376 patients with essential hypertension of varying severity revealed that the milder hypertensive patients lived longer. If they died of causes related to hypertension, they usually succumbed to coronary disease, congestive heart failure, and cerebrovascular accidents. Patients with malignant hypertension lived a shorter period and died in uremia. The incidence of significant coronary sclerosis was highest in the milder hypertensive patients and lowest in the patients with malignant hypertension. Associated renal impairment was definitely correlated with the severity of the hypertension. Although correlation existed between the weight of the heart and the severity of the hypertension, no correlation occurred between the weight of the heart and the duration of known hypertension.

HARRIS

Thorpe, J. J., Welch, W. J., and Poindexter, C. A.: Bilateral Thoracolumbar Sympathectomy for Hypertension. Am. J. Med. **9:** 500 (Oct.), 1950.

The authors studied the course of 500 hypertensive patients subjected to bilateral thoracolumbar sympathectomy over a six year period. Comparison of the survival curves of this group with a comparable group treated medically at the end of three years showed no significant difference in groups 1, 2, and 3. A definite prolongation of life in the advanced cases of hypertension (group 4) was found in the operated cases.

The original Smithwick procedure is the operation of choice, since the degree of blood pressure reduction and the subjective improvement obtained with more extensive procedures do not justify the increased morbidity, postoperative complications, and higher mortality rate. Thoracolumbar sympathectomy is indicated as a palliative procedure for hypertensive patients in group 4 and for selected cases in group 3. Contraindications to operation include intractable cardiac failure, renal insufficiency with urea nitrogen above 20 mg. per cent, evidence of mental confusion, history of a cerebrovascular accident, myocardial infarction less than six months before operation, and a history of a serious psychiatric disturbance at any pervious period.

HARRIS

Judson, W. E., Epstein, F. H., Wilkins, R. W.: The Comparative Effects of Small Intravenous Doses of l- Nor-epinephrine Upon Arterial Pressure and Pulse Rate in Normotensive Subjects and Hypertensive Patients before and after Thoracolumbar Sympathectomy. J. Clin. Investigation **29:** 1414 (Oct.), 1950.

The authors found in a previous study that no significant difference existed between the blood pressure response to l-norepinephrine between normotensive subjects and in hypertensive patients before sympathectomy.

Thoracolumbar sympathectomy did not strikingly alter the pressor response of hypertensive patients to intravenous norepinephrine, although some hypertensives soon after splanchnicectomy may show increased sensitivity to the smaller doses.

However, the pulse rate response, which was a definite bradycardia in normotensives, failed to occur in the preoperative hypertensive group. Following thoracolumbar sympathectomy the pulse rate of hypertensives slowed normally during the hypertensive response to intravenous norepinephrine.

WAIFE

Danford, H. G., Dieter, D. G., Christofferson, J. W., and Herrin, R. C.: Effect of Dietary Restriction of Salt and Protein on Blood Pressure of Hypertensive Rats. Am. J. Physiol. **163:** 190 (Oct.), 1950.

Rats were rendered hypertensive by means of a silk perinephritis. Animals on low (0.0 to 0.05 per cent) sodium chloride had pressures which declined 15 to 51 mm. Hg. after 40 days. Diets with lowest sodium had the greatest hypotensive effect. Natural sources of food as rice or potato made no difference. The diets studied lowered pressure without any significant loss in body weight. Casein (4 to 18 per cent) was without effect on the hypotensive response to salt restriction. Twice as many rats with hypertension survived 220 days postoperatively on low sodium diets as did hypertensive controls. Since removal of the sick capsule lowered pressure in 3 of 4 animals, and Etamon chloride or surgical anesthesia with Nembutal failed to do so, the authors concluded that hypertension in these animals depended on renal factors rather than nervous ones.

OPPENHEIMER

Shumacker, H. B.: Transpleural and Extraperitoneal Approach for Extensive Sympathectomy and Splanchnicectomy. Surg., Gynec. & Obst. **91:** 711 (Dec.), 1950.

The author describes technics for the performance in two stages of total, or less complete but extensive, sympathectomy and splanchnicectomy by use of the transthoracic and extraperitoneal approaches. The procedures described have been carried out 55 times in 30 hypertensive patients whose mean age was 42 years. The author strongly favors the surgical approach described because of the adequacy with which the nerves are exposed, the direct ease with which any vessels which might prove troublesome can be dealt with, the exactness with which the resection can be carried to the desired extent, and the generally good manner in which the procedure has been tolerated. These operations are attended by a reasonably low mortality and morbidity.

BECK

Moses, C., Longabaugh, G. M., and George R. S.: Production of Hypertension Following Choline Deficiency in Weanling Rats, Proc. Soc. Exper. Biol. & Med. **75:** 660 (Dec.), 1950.

The authors performed this study to determine the effect upon the blood pressure at maturity of varying degrees of choline deficiency for a five day period after weaning and to observe the effect of a similar choline deficiency on more mature rats.

Twenty out of 28 (71 per cent) of weanling rats, surviving a five-day choline-deficient diet and maintained on a normal diet for six months thereafter developed arterial blood pressure levels of over 150 mm. Hg. Hypertension did not develop in the animals fed 0.4 mg. or 2.0 mg. of choline. Similarly, hypertension did not develop in the group of older rats fed either a choline free or low choline diet for 10 days and then a normal diet for six months.

MINTZ

Hilden, T.: Hypertensive Encephalopathy Associated with Hypochloremia. Acta. med. Scandinav. **136:** 199, 1950.

In 5 patients hypertension and acute cerebral disturbances were associated with transient hypochloremia and increases in blood urea. There was no evidence of chronic glomerulonephritis although chronic pyelonephritis was present in 2 cases. Vomiting was probably not the cause of the plasma hypochloremia (230 to 310 mg. per 100 ml.) because some vomited little or not at all and the plasma carbon dioxide values were normal. Subcutaneous administration of saline seemed to improve their condition.

WAIFE

Govaerts, J., and Regnier, M.: Results of Surgical Treatment of So Called "Essential Hypertension". Acta cardiol. **5:** 473, 1950.

Abdominal sympathectomy had a beneficial effect only in patients belonging to groups 0, I and II of Keith, Barker, and Wagener and was effective in group III when preoperative tests indicated the presence of a vasospastic factor and only moderate impairment of renal function. Extensive dorsolumbar sympathectomy should be preferred to abdominal extirpation because the late effects are more favorable, particularly in patients belonging to groups III and IV. Group IV, however, should be carefully selected with elimination of cases with greatly impaired renal function. Surgical treatment is justified even in the presence of a slight degree of heart failure.

Renal biopsy during operation permits a fair estimation of the results and a relatively exact prognosis for the future. The more favorable results are obtained when renal alterations are not yet marked. Changes in the retinal vessels correspond roughly to changes observed in the kidneys, but may occur earlier or later than the renal alterations.

Although surgical treatment does not settle the problem of hypertension it brings about an appreciable fall, and protects against sudden increase, of the arterial pressure. Thus, it may prevent a functional, neurogenic hypertension from becoming an irreparable condition with organic, sclerotic vascular changes.

PICK

PATHOLOGIC PHYSIOLOGY

Hamilton, W. F., Remington, J. W., and Hamilton, W. F., Jr.: Factors Relating to Heart Size in the Intact Animal. Am. J. Physiol. **163:** 260 (Nov.), 1950.

The heart size of intact dogs is correlated with the duration of diastole, mean systolic pressure, stroke volume and work per beat. When epinephrine is infused intravenously, hearts are large and slow with high mean systolic pressures. Stroke volumes and work per beat are less than in controls. Correlation between mean systolic pressure and heart size is set by the fact that when pressures are high the duration of diastole is long. After vagotomy, epinephrine infusion produces a rapid small heart associated with high mean systolic pressures. Stroke volumes and work continue to be less than in controls. Bleeding does not change the differences. At high normal venous pressures, reflex changes in diastolic time are parallel to changes in venous pressure and heart size. When venous pressure is very low the heart is large or small, depending on diastolic filling time. When duration of diastole is very short the venous pressure is determined by the venous return and may be high or low. The most important factor in fixing the diastolic size of the heart, in the intact animal, is the filling time as determined by the heart rate.

OPPENHEIMER

Chambliss, J. R., Demming, J., Wells, K., Cline, W. W., and Eckstein, R. W.: Effects of Hemolyzed Blood on Coronary Flow. Am. J. Physiol. **163:** 545 (Dec.), 1950.

The authors report that a potent vasodilator substance is released when small amounts of whole blood are rapidly injected through a small bore needle into a cannulated coronary artery. Increases in coronary flow from 50 per cent to slightly over 100 per cent resulted. These increases were not accompanied by changes in arterial or perfusion pressures. The duration of effect was 5 to 30 seconds. Whole blood slowly injected was without significant effect. Saline or serum rapidly injected increased coronary blood flow. Laked blood has similar properties. The authors demonstrated that forcible injection through a small bore needle traumatized red blood cells. Blood traumatized in a beaker increased coronary blood flow when slowly injected. Hemolysis of red cells is the common factor in all injections tested. The vasodilator material (adenosine-triphosphate–like) released, is active in small concentrations. The need for critical evaluation of data from experiments making use of extracorporal circulation of blood through long perfusion tubes, squeeze type pumps, differential flow meters, and other types of mechanical apparatus is emphasized.

OPPENHEIMER

Nickerson, M. and Nomaguchi, G. M.: Blockade of Epinephrine-Induced Cardioacceleration in Frog. Am. J. Physiol. **163:** 484 (Dec.), 1950.

The authors conclude that the chronotropic response of the frog heart to epinephrine produces a stimulation of utilizable acetate. This may proceed by a stable pathway, which is constant the year round and is blocked by β-haloalkylamine and adrenergic blocking agents, or a labile pathway present normally only in summer frogs, which is activated by anterior pituitary gland and blocked by fluoroacetate or iodoacetate. A second mechanism involved in the chronotropic response to epinephrine involves a trigger action which is effective only in the presence of a suitable acetyl substrate. Both pathways appear to be independent of energy sources utilized in the normal contraction of the heart and in its positive inotropic response to epinephrine. Epinephrine thus appears to promote production of the metabolic substrate required for the expression of its own chronotropic action.

OPPENHEIMER

Jourdan, F., Heyraud, J., and Collet, A.: Influence of the Vagal Nerves upon Experimental Nodal Rhythm. Compt. rend. Soc. de Biol. **144:** 1067 (Aug.), 1950.

The authors produced persistent nodal rhythm in 5 rabbits by extirpation of the sinus node, and studied the effects of electrical stimulation of the peripheral stumps of the cut vagus nerve. Stimulation on the right as well as on the left side had a marked chronotropic effect, and produced extreme slowing of the heart rate to complete and prolonged standstill of the whole heart. A negative dromotropic effect was demonstrated in electrocardiograms, which showed a marked increase of the shortened P-R interval, and occasional development of second degree A-V block with a 2:1 or even higher ratio. With stimulation for more than 10 seconds escape of a slow rhythm was observed, which in the electrocardiogram still showed the criteria of an A-V nodal rhythm.

PICK

Leeds, S. E.: A Cannula for Simultaneous Drainage of Both Cavae in Artificial Heart Experiments. Proc. Soc. Exper. Biol. & Med. **78:** 468 (Nov.), 1950.

The authors constructed a single right angle cannula which permits blood from both the cavae to pass through it and thence flow to the pump. The cannula consists of thin walled (26 gage) metal tubing of uniform diameter with two arms at an angle of 90 degrees. The tube is open at both ends and is also open at the vertex of the angle. With the cannula ligated in place, no blood from the cavae can enter the right auricle, and the blood must be diverted. The right auricle or ventricle may be opened with very little blood loss since only the coronary flow enters the operative site. The coronary flow constitutes about 5 or 6 per cent of the cardiac output.

The cannula was used successfully in 19 experiments in which the extracorporeal pump was employed. The entire venous flow of blood was diverted for a period of 13 to 68 minutes followed by survival of the animals for an indefinite period.

MINTZ

Blake, W. D., Wegria, R., Ward, H. P., and Frank, C. W.: Effect of Renal Arterial Constriction on Excretion of Sodium and Water. Am. J. Physiol. **163:** 422 (Nov.), 1950.

A Goldblatt clamp was placed about the right renal artery and the left kidney served as a control. Minimal constriction of the renal artery with no detectable decrease in glomerular filtration rate was accompanied by decreased urine flow and sodium excretion. This depended on increased sodium reabsorption in at least two experiments. Intrarenal mechanisms other than filtration rate which may affect sodium reabsorption and excretion are suggested, such as concentration of sodium in plasma and rate of flow of urine through tubules.

OPPENHEIMER

PATHOLOGY

Askey, J. M.: Spontaneous Rupture of a Papillary Muscle of the Heart. Review with Eight Additional Cases. Am. J. Med. **9:** 528 (Oct.), 1950.

The writer reviews 37 cases of spontaneous rupture

of a papillary muscle of the heart, including 8 new cases. This condition usually follows myocardial infarction, but may occur with necrotizing periarteritis, endocarditis, sepsis, and syphilis. The antemortem diagnosis of a ruptured papillary muscle of the heart or of a ruptured interventricular septum should be considered when a cardiac murmur suddenly appears or a previous murmur is intensified. The murmur is usually systolic in a rupture of the interventricular septum, but may be both systolic and diastolic in a rupture of a papillary muscle of the heart. A thrill, frequently present in a rupture of the interventricular septum, is absent in a rupture of the papillary muscle.

HARRIS

Reingold, I. M.: Myocardial Lesions in Disseminated Coccidioidomyocosis. Am. J. Clin. Path. **20:** 1044 (Nov.), 1950.

Of 4 cases of disseminated coccioidomycosis, nonspecific myocardial changes were found in three. In only 1 case did specific myocardial lesions occur. The lesions consisted of small foci of necrosis with infiltration of mononuclear cells, lymphocytes, and Langhans-type giant cells.

HANNO

Rodriguez, C. E., Wolfe, A. L., and Bergstrom, V. W.: Hypokalemic Myocarditis: Report of Two Cases. Am. J. Clin. Path. **20:** 1050 (Nov.), 1950.

Autopsy of 2 cases of diabetic acidosis disclosed focal necrosis and lymphocytic infiltration of the myocardium. Because similar lesions have been reported in animal and human subjects with potassium deficiency, and because hypopotassemia occurs in diabetic acidosis, the authors conclude that the myocarditis found in their cases is hypokalemic in causation.

HANNO

Nothacker, W. G., and Netsky, M. G.: Myocardial Lesions in Progressive Muscular Dystrophy. Arch. Path. **50:** 578 (Nov.), 1950.

The authors report on the myocardial lesions in progressive muscular dystrophy. They found lesions similar to those found regularly in skeletal muscle in 6 of 11 cases. Both simple and pseudohypertrophic types of the disease showed myocardial involvement. Two of the 6 cases exhibited an irregular heart action. There was no electrocardiographic study and clinical data was meager. At necropsy, the myocardium throughout both ventricles was infiltrated with grey scars. The intervening muscle was pale, in one case yellow, and in most instances presented, on cross section, a coarse appearance which was due to irregularly entwining scar tissue. Microscopically, some of the scars contained groups of fat cells; many of the isolated or trapped myocardial fibers contained fat vacuoles. The coronary arteries were normal in all cases, as were the valves.

Death in most instances was due to terminal pneumonia. In one case, it was due to heart failure, autopsy revealing bilateral hydrothorax and ascites.

GOULEY

PHARMACOLOGY

Hendricks, J. P.: Contribution to the Study of Vasodilating Drugs. Acta clin. belg. **5:** 309 (July–Aug.), 1950.

The author describes a very sensitive device (based on the principle of the photoelectric cell) for the measurement of volume variations of the pulse wave, which he used in a study of the effect of various vasodilating agents in normal persons and in patients with impaired peripheral circulation.

Intravenous injection of nicotinic acid or of 2-benzyl-4,5-imidazoline chloride produced a marked, but transitory, increase of the volume pulse in various parts of the body. Intravenous injection of β-pyridyl carbinol had a more permanent vasodilating effect, mainly on the cerebral circulation, and had no side effects such as were observed following administration of nicotinic acid.

PICK

Burch, G., Ray, T., Threefoot, S., Kelly, F. J., and Svedberg, A.: The Urinary Excretion and Biologic Decay Periods of Radiomercury Labeling a Mercurial Diuretic in Normal and Diseased Man. J. Clin. Investigation **29:** 1131 (Sept.), 1950.

Mercuhydrin labeled with radioactive mercury was administered parenterally to 83 hospitalized subjects. When cardiovascular and renal functions are normal, about one-half of the mercurial diuretic administered intramuscularly is excreted in 1 to 8 hours (average about 3 hours). Excretion of the intravenous diuretic was somewhat more rapid.

Congestive heart failure results in delay in excretion of mercury. The state and phase of the failure influenced the rate of excretion and individual variations were large.

Subjects with impaired renal function retained considerable quantities of radiomercury even though urinary volumes were relatively high. The degree of impairment may be great enough to lead to a toxic accumulation of mercury on succesive administration. One subject with renal insufficiency excreted only 2.5 per cent of injected mercury in about four and one-half days. There is a need for continual evaluation of renal function to ascertain whether mercury is being retained in excessive quantities.

WAIFE

Coombes, A. C. A., and Roche, E. H.: A Fatal Case of Delayed Hypersensitive (Anaphylactic) Reaction to a Test Dose of Vasiodone. Brit. Heart J. **12:** 360 (Oct.), 1950.

The authors describe a case of delayed, fatal anaphylactic reaction to a small test dose of Vasio-

done, a radiopaque dye. They attribute this to the administration of an antihistaminic drug, which so delayed the sensitivity reaction as to give an apparently negative result when there was, in fact, extreme sensitivity. The authors state that antihistamine drugs should be used in every case, but an hour before the main dose of the radiopaque substance. The latter should not be used at all in individuals with a strong allergic history, and , whenever used, very small initial test doses should be given.

SOLOFF

Mosey, L., and Stutzman, J. W.: The Effect of Cyclopropane, Ether, and Thiopental Sodium upon the Over-Digitalized Heart. Proc. Soc. Exper. Biol. & Med. **75:** 34 (Oct.), 1950.

Dogs, digitalized to cardiac toxicity with ouabaine (0.07 to 0.08 mg. per Kg.) or with digitoxin (0.3 mg. per Kg.) were anesthetized with cyclopropane, ether and thiopental sodium. Under cyclopropane anesthesia 8 out of 10 dogs showed improvement or the abolition of the digitalis induced arrhythmias. Two dogs showed no improvement. All dogs demonstrated a return to, or maintenance of, pre-anesthetic abnormalities upon recovery from anesthesia. The increase in heart rate with cyclopropane in all dogs but one suggests a decreased vagal tone. This reduction in vagal tone combined with increased irritability of supraventricular tissues may explain the counteraction of digitalis arrhythmias by cyclopropane.

Ether caused a reversion of the arrhythmias to a normal rhythm in all but one dog. The mechanisms responsible for the increased heart rate with ether anesthesia are: paresis of the vagal inihbitory mechanism, augmentation of the cardiosympathetic impulses and liberation of certain hormones such as epinephrine or sympathin. Thiopental sodium exerted no constant effect upon the electrocardiographic abnormalities of overdigitalized dogs.

MINTZ

Gordon, A. J., Brahms, S. A., Megibow, S., and Sussman, M. L.: An Experimental Study of the Cardiovascular Effects of Diodrast. Am. J. Roentgenol. **64:** 819 (Nov.), 1950.

In a series of nineteen experiments upon 15 adult dogs which received from two to four times as much Diodrast per unit of weight as the human subject, the following effects were noted: a rise in pulse pressure due chiefly to a fall in the diastolic pressures; changes in the contour of the femoral pulse tracing consisting of a more rapid rise, loss of apical rounding, a more rapid fall, loss of the dicrotic wave, and increase in the height of the wave; a rise in the venous pressure; and a fall in the pulse rate. The same results were obtained, but to a lesser degree, when a 35 per cent solution of Diodrast was substituted for the 70 per cent solution.

The typical Diodrast reaction could be modified by changes in the speed of injection, but not eliminated, even when the injection was given slowly. Significant effects of the drug injection were vasodilatation, and stimulation followed by depression of the myocardium. Abdominal compression, epinephrine, ephedrine, pitressin, atropine, vagotony, tetraethyl ammonium and an antihistaminic failed to prevent the vasodepression.

No evidence of allergic sensitization occurred in dogs which received two injections of Diodrast several weeks apart.

SCHWEDEL

Zeh, E.: Comparative Investigations on the Effect of Gynergen and Hydrergin on the Electrocardiogram. Contribution to the Application of the Ergot Test. Ztschr. f. Kreislaufforsch. **39:** 673 (Nov.), 1950.

The authors treated 50 patients with electrocardiograms typical of neurocirculatory asthenia with intramuscular injections of 0.5 mg. Gynergen, and after an interval of two to three days, by injection of 0.3 mg. Hydrergin. The effect of the two drugs on the abnormal electrocardiogram was followed for about an hour. Hydrergin produced a more marked slowing of the heart rate, while normalization of the abnormal ST-T occurred more readily following Gynergen. Since the latter drug is known to produce coronary constriction, the normalization of the electrocardiogram cannot be explained by alteration of the coronary circulation and is probably due to changes in the muscular tone of the heart.

PICK

Bender, C. E., Hoxsey, R. J., and DeMarsh, Q. B.: Neutropenia in a Patient Treated with a Mercurial Diuretic and Its Response to BAL. Ann. Int. Med. **33:** 1285 (Nov.), 1950.

Following administration of 256 cc. of mercupurin over a ten month period for treatment of congestive failure, a 61 year old white woman was admitted to the hospital in a lethargic state. Blood studies showed leukopenia, and sternal marrow examination revealed a marked maturation arrest of the myeloid series. The latter, ascribable to depression of the bone marrow by the mercurial compound, was apparently responsible for the changes in the peripheral blood. Following administration of BAL for five successive days, the white blood count in the peripheral blood returned to normal, but the patient ultimately succumbed as a result of progressive heart failure.

WENDKOS

Cunningham, G. C., and Schnitzker, W. F.: The Use of Neo-Synephrine in Paroxysmal Supraventricular Tachycardia. J. Pediat. **37:** 727 (Nov.), 1950.

The authors report a case of a 27 month old white male child with paroxysmal auricular tachycardia,

probably due to a congenital cardiac defect. On one occasion the tachycardia failed to respond to carotid sinus pressure, eyeball pressure, or prostigmine, but did respond to 0.2 mg. of acetylcholine bromide. During another attack, acetylcholine bromide also failed and the patient was controlled on combined digitalis and quinidine therapy. Intravenous Neosynephrine was of immediate benefit each time it was used during the last three episodes. The authors recommend the initial dose of 0.1 mg., which can be increased by 0.1 mg. at intervals of thirty minutes to as high as 0.5 mg.

MARGOLIES

King, J. T.: Digitalis Delirium. Ann. Int. Med. **33:** 1360 (Dec.), 1950.

The author reports that in six patients, ranging in age from 33 to 62, delirium occurred in association with digitalis therapy. In each case, the disturbance cleared entirely on withdrawal of the drug or reduction in its dosage. Deficiency in cerebral circulation may play a predisposing role, but it is hazardous to place too much emphasis on arteriosclerosis or age. Three of the cases reported had aortic valve lesions, probably on a rheumatic basis. The delirium seemed to be due to drug intoxication, rather than to changes in circulation or absorption of edema products in these cases. Causative preparations included powdered leaf of digitalis, lanatoside C and digitoxin.

WENDKOS

McCormick, R. V.: Periarteritis Occurring During Propylthiouracil Therapy. J. A. M. A. **144:** 1453 (Dec.), 1950.

The author reports the first case of fatal generalized periarteritis caused by propylthiouracil. An elderly woman was given 50 mg. of propylthiouracil four times daily for 10 days, then reduced the dose herself to one-half the recommended amount because of gastric upset. After five more days she stopped the drug altogether. On her next return to the clinic she was admitted to the hospital and after further studies she was again given propylthiouracil. Within 24 hours her temperature rose, diarrhea developed, and later the patient became incontinent and showed signs of cerebral difficulty. The drug was stopped and within 24 hours the patient was greatly improved. One week later she was given a single dose as a test, and she again developed the original signs and symptoms. Two months later in the out patient clinic she was inadvertently started again on propylthiouracil. After 1,150 mg. of the drug was taken in six days she was brought to the hospital in an unconscious condition and died six days after admission. Postmortem studies disclosed diffuse periarteritis.

KITCHELL

Malinow, M. R., Moia, B. and Manguel, M.: The Effect of Oxygen Therapy Determined by Measurement of Oxygen Consumation. Acta Cardiol. **5:** 457, 1950.

In order to estimate the benefit of oxygen therapy, the authors studied oxygen consumption, pulmonary ventilation and oxygen utilization before and during inhalation of pure oxygen in 32 subjects. The cases were divided into four groups, normal subjects, patients with coronary insufficiency in various stages, patients with congestive failure, and patients with coronary disease and congestive failure.

No significant changes of oxygen consumption which could be attributed to oxygen inhalation could be demonstrated, and the same small variations occurred in normal subjects as well as in patients with heart disease. A slight increase of utilization of oxygen, found in some cases during the inhalation of the gas, could be correlated with a simultaneously occurring decrease of pulmonary ventilation.

The authors conclude that a beneficial effect of inhalation of pure oxygen could not be demonstrated objectively in the studied groups of patients.

PICK

PHYSICAL SIGNS

Bishop, L. and Logue, B.: External Rupture of the Heart Causing a Systolic Murmur and Thrill. J. A. M. A. **144:** 757 (Oct.), 1950.

The authors report the case of a 69 year old woman who, fifteen minutes prior to admission to the hospital, had severe crushing substernal pain which radiated to the shoulder and back. Blood pressure at this time was 160 systolic and 100 diastolic in both arms. Electrocardiographic tracing showed early changes of acute anterior myocardial infarct. Three hours later it was noted that the blood pressure had dropped to 90 systolic and 60 diastolic and an intense, harsh systolic murmur with a purring systolic thrill over the lower part of the sternum and to the left of the sternum in the fourth and fifth intercostal spaces was present. A clinical diagnosis of rupture of the interventricular septum was made at this time. She died suddenly about two and one-half hours after signs of rupture of the heart were detected. Postmortem examination showed a ragged longitudinal tear 1.2 cm. in length extending through all coats of the heart wall which was thin and friable, measuring only 0.8 cm. in thickness. The interventricular septum was not involved. This case is of interest because rupture of the heart occurred only four hours after the clinical infarction and softening must have taken place extremely rapidly. It is difficult to explain the murmur and thrill in such circumstances. Possibly it was due to the fact that the defect in the external wall was small in systole and a jet of blood was expelled under high pressure, and in diastole some of this reentered the ventricle producing a murmur and thrill simulating that of rupture of interventricular septum.

KITCHELL

PHYSIOLOGY

Lewis, A. E., and Goodman, R. D.: The Excretion Kinetics of the Dye T-1824 in Relation to Plasma Volume Determinations. J. Lab. & Clin. Med. **36:** 599 (Oct.), 1950.

Since a high degree of accuracy is desirable in the determination of plasma volume, the authors studied the kinetics of T-1824 (Evans blue) disappearance by a constant infusion method designed to eliminate the variable factor of mixing time. This method also makes it possible to estimate the amount of dye lost from the body.

The calculation of plasma volume is based on based on rates of change in plasma concentration and, therefore, the effects of mixing time are eliminated. In rabbits the error produced by neglecting mixing time is negligible.

The data indicate that in rabbits there is no significant difference in calculating plasma volumes of linear or exponential extrapolation. The excretory rate of T-1824 in rabbits is so low that estimations of clearance or maximum excretory rate are invalidated by relatively small experimental errors.

MINTZ

Orias, O., Brooks, C. McC., Suckling, E. E., Gilbert, J. L., and Siebens, A. A.: Excitability of the Mammalian Ventricle throughout the Cardiac Cycle. Am. J. Physiol. **163:** 272 (Nov.), 1950.

When new technics are used it can be shown that stimuli of intermediate or long duration near maximal strength (20–30 ma.) often produce a response during a 10 millisecond interval early in the cycle but fail to do so when placed a few milliseconds later. Ten to twenty milliseconds later still in the cycle maximal stimuli are again effective. In these experiments it is characteristic of the recovery process that the muscle periodically attains a degree of excitability that is not sustained. Periods of reduced excitability precede further recovery. Usually only one major "dip" or period of relatively increased excitability is present in the refractory phase of the cycle.

OPPENHEIMER

Freed, S. C., and Friedman, M.: Hypotension in the Rat following Limitation of Potassium Intake. Science **112:** 788 (Dec.), 1950.

The authors report that rats maintained on a very low potassium diet developed a profound hypotension, a marked degree of generalized flaccidity of the skeletal muscles, and retarded growth. However, other rats on an identical diet, but supplied with potassium, maintained a normal blood pressure. Another group of animals on a standard diet, but partially starved, grew as slowly as those in the experimental group, but had a normal blood pressure. The authors believe that the hypotension observed was due to a specific potassium deficiency.

WAIFE

Ring, G. C., Sokalchuk, A., Navis, G. J., and Rudel, H. W.: Positional Changes of the Heart and Their Effects on Electrokymographic Recordings. Am. J. Physiol. **163:** 475 (Dec.), 1950.

The authors point out the distortions which positional changes produce in the dog electrokymogram. In the left lateral position, the heart moves toward the sternum and the densograms taken in sternal areas are too small and in the spinal areas are too large. Near the middle of the heart the timing and probably the amplitude of the inward movements are correctly represented. The positional movements of the human heart are smaller and the densograms are less distorted by these movements. The large vessels also show positional changes and therefore electrokymographic records taken from these for timing of cardiac events must be used with caution.

OPPENHEIMER

Smith, H. L., Essex, H. E., and Baldes, E. J.: A Study of the Movements of Heart Valves and of Heart Sounds. Ann. Int. Med. **33:** 1357 (Dec.), 1950.

The authors perfused the hearts of various animals with oxygenated Ringer-Locke solution and kept them beating for various lengths of time. Rather large openings were made in the walls of the auricles and a sound recording, visual record of the sound and an electrocardiogram were made at the same time that motion pictures were taken. It was observed that the auriculoventricular valves did not close when there was little or no fluid in the ventricles, and would close when there was sufficient fluid in the ventricles. It was noted that there was a very definite sphincter-like action of the mitral and tricuspid rings. Experiments were done which indicate that the first heart sound is due largely to the valves suddenly becoming taut and striking forcefully against each other; the forceful striking of the edges of the valves against each other produce a much louder sound than does the sudden tautening of the valves, such forceful striking being the main factor in producing the first heart sound.

WENDKOS

RHEUMATIC FEVER

Brick, M., McKinley, H., Gourley, M., Roy, T. E., and Keith, J. D.: Oral Penicillin Prophylaxis in Rheumatic Fever Patients. Canad. M. A. J. **63:** 255 (Sept.), 1950.

Thirty-eight children with rheumatic fever received 50,000 units of penicillin orally twice a day during the fall, winter and spring over a two year period. An equal number of comparable subjects served as controls. Of 576 throat cultures in the untreated children, fifty-two were positive for beta hemolytic streptococci as compared with only three positive cultures out of 570 in the penicillin-treated group. Penicillin had no effect on the incidence of

nonstreptococcal upper respiratory tract infection. Recurrences of rheumatic fever developed in 6 control and 3 treated subjects. While the authors feel that there was some suggestive evidence that prophylactic oral penicillin was useful in preventing recurrances of rheumatic fever, they believe that the almost complete elimination of hemolytic streptocci from the throat suggests the use of prophylactic oral penicillin during epidemics of scarlet fever or streptoccal sore throats.

WAIFE

Maliner, M. M.: Oral Penicillin in the Prophylaxis of Recurrent Rheumatic Fever. J. Pediat. **37:** 858 (Dec.), 1950.

Sixty-three children with a definite history of rheumatic fever and 23 children with congenital heart disease were examined once a month from September through June. Routine throat cultures, sedimentation rates and vital capacities were done at each visit. Thirty-three of the rheumatic children and 23 of the congenital group received penicillin troches of 5,000 units three times daily. The remainder served as controls, receiving placebo troches of similar size, shape and taste.

In the control group of 30 rheumatic children, 2 had recurrences of rheumatic fever; in neither of these cases, however, was a culture of *Streptococcus hemolyticus* present. In the group of 33 rheumatic children receiving penicillin troches, there were no cases of rheumatic activity. None of the cases developed subacute bacterial endocarditis. The author concludes that penicillin troches are of value in temporarily eliminating *Str. hemolyticus* from the throats of rheumatic children. Because of the dangers involved in the use of the sulfonamides, the author suggests that penicillin troches replace the sulfonamides in rheumatic fever control programs.

MARGOLIES

Copeman, W. S. C., and Pugh, L. G. C. E.: Dehydration Treatment of Rheumatic Fever. Lancet **259:** 765 (Dec.), 1950.

To test the validity of the theory of Reid and others that the effectiveness of salicylate therapy in acute rheumatic fever is due to cellular dehydration, the authors dehydrated 7 patients with rheumatic fever, using severe fluid restriction, sodium sulfate purges and 50 cc. of a 30 per cent solution of sodium chloride intravenously. This resulted in relief of joint pain on movement, tenderness and swelling, and a fall in temperature and pulse rate. The sedimentation rate remained unchanged three days after therapy. The authors point out the similarity between the effectiveness of salicylates and dehydration therapy but conclude that other factors than alteration in the volume and distribution of body fluids may play a role, for example, nonspecific stimulation of the adrenal cortex.

BERNSTEIN

ROENTGENOLOGY

Goodwin, W. E., Scardino, P. L., and Scott, W. W.: Translumbar Aortic Puncture and Retrograde Catheterization of the Aorta in Aortography and Renal Arteriography. Ann. Surg. **132:** 944 (Nov.), 1950.

The authors discuss features of the technic and application of translumbar aortic puncture. The aorta is punctured usually at the level of the first lumbar vertebra with a spinal needle 6 inches long, 16 or 17 gage in adults, and 20 gage in children. They describe its use, which resulted in the demonstration of multiple arteriovenous anastomoses and a large multiloculated aneurysm of the hypogastric artery.

Retrograde catheterization was accomplished through a branch of the profunda femoris under direct surgical exposure. A No. 10 F catheter is passed upwards through the femoral artery to the desired level in the aorta. Thirty cc. of the opaque dye is injected and suitable roentgen exposures are made. Femoral artery catheterization was carried out fifteen times in 14 patients. An aneurysm of the thoracic aorta below the site of coarctation, and atherosclerotic plaques in major branches of the aorta could be demonstrated.

SCHWEDEL

Leigh, T. F., and Rogers, J. V.: Visualization of the Abdominal Aorta and its Branches following Intravenous Injection of Contrast Medium. Am. J. Roentgenol. **64:** 945 (Dec.), 1950.

Four cases are presented in which the abdominal aorta and its branches were visualized during angiography. In one, trauma resulted in a lack of opacification of the left renal artery and kidney, and 30 minutes later a lack of renal pelvic opacification. Visualization of the abdominal aorta in the second case indicated obstruction at the level of the renal arteries, the left renal artery and kidney failed to opacify; again, the renal pelvis did not opacify one half hour later. This was corroborated at necropsy. Two other patients were subjected to this procedure. An aortic obstruction below the renal arterial level was found in one; the other was normal.

SCHWEDEL

SURGERY IN HEART AND VASCULAR SYSTEM

Weiss, E. W., and Lam, C. R.: Tantalum Tubes in the Non-Suture Method of Blood Vessel Anastomosis. Am. J. Surg. **80:** 452 (Oct.), 1950.

The authors experimented with dogs using tantalum tubes to reestablish the continuity of sectioned femoral arteries. In some instances the tubes were treated with a coating of paraffin, while in others they were lined with a segment of corresponding vein.

The unlined tubes and those coated with paraffin

were all occluded by thrombi. However, two-thirds of the tubes lined with veins were found to be patent when examined at intervals varying from four hours to two months after operation. The best results were obtained with the use of vein grafts placed between two short tantalum cuffs.

According to the authors, tantalum is as good as vitallium or any other material which has been used to reestablish continuity of vessels.

ABRAMSON

Swan, H., Maaske, C., Johnson, M., and Grover, R.: Arterial Homografts. II. Resection of Thoracic Aortic Aneurysm Using a Stored Human Arterial Transplant. Arch. Surg. **61:** 732 (Oct.), 1950.

The authors report experimental data suggesting that, in general, grafts stored for less than 40 days are preferable to those stored for much longer periods, although the latter give satisfactory short term results. The many practical problems related to the establishment of an artery bank and the limitations on the use of homogeneous arterial grafts, particularly in regard to the size and length of the transplant, are discussed. A case report is presented in which an adult type of coarctation of the aorta together with a mycotic aneurysm of the descending aorta in a 16 year old boy was resected and the gap in the aorta bridged by an 8 cm. human arterial homograft, which had been stored for 52 days. Six months after operation the vessel was apparently functioning well.

BECK

Gurdjian, E. S., Webster, J. E., and Martin, F. A.: Carotid-Internal Jugular Anastomosis in the Rhesus Monkey. Angiographic and Gasometric Studies. J. Neurosurg. **7:** 467 (Nov.), 1950.

The authors describe the historical background of carotid-internal jugular anastomosis. Shunts were performed on Rhesus monkeys. Angiographic and gasometric studies show that following these shunts, the blood does not flow into the sagittal, straight or petrosal sinuses for distribution by retrograde flow. Instead it seeks a ready exit via the neck veins, the basilar veins and the lateral sinuses. The shunted flow can be directed into the intracerebral veins by ligation of the opposite lateral sinus under high pressures. In animals with the opposite lateral sinus ligated, the oxygen volume per cent of the sagittal sinus blood is not increased. There appears to be a stagnation of the blood in the sagittal sinus following the shunts.

BECK

Grant, J. L., Fitts, W. T., Ravdin, I. S.: Aneurysm of the Hepatic Artery: Report of Two Cases and a Consideration of Surgical Treatment. Surg., Gynec. & Obst. **91:** 527 (Nov.), 1950.

Aneurysm of the hepatic artery, although a rare cause of biliary symptoms, must be considered in the differential diagnosis of jaundice. Hepatic aneurysm produces pain, jaundice, and bleeding into the gastrointestinal tract. Heretofore, the diagnosis has rarely been made before death and only 14 patients with aneurysm have been operated on.

Two additional hepatic aneurysms, both diagnosed at operation, are reported. Both patients died, one from massive hepatic necrosis, the other from recurrent hemorrhage.

The only reported cures of hepatic aneurysm have been achieved by ligation of the artery. Several recent developments may improve the results of treatment, although to our knowledge they are still untested in humans: (1) the use of antibiotics in the prevention of liver necrosis following ligation; (2) reconstruction of the artery by grafts of artery or vein; and (3) anastomosis of the ligated hepatic artery to the portal system.

BECK

AMERICAN HEART ASSOCIATION, INC.

1775 BROADWAY, NEW YORK 19, N. Y.

Telephone Plaza 7-2045

ANNUAL ELECTIONS

Officers

Dr. Louis N. Katz, Chicago, has assumed the Presidency of the Association for the 1951–52 term. Dr. Katz is Director of Cardiovascular Research, Michael Reese Hospital, and Professorial Lecturer in Physiology, University of Chicago. He also has served as Chairman of the Association's Research Committee. Dr. Katz succeeds Dr. Howard B. Sprague, Brookline, Mass.

At the Twenty-seventh Annual Meeting of the Association in Atlantic City last month,

Dr. Irving S. Wright, New York, was chosen President-Elect for the 1952–53 term. Dr. Wright is Professor of Clinical Medicine, Cornell University, and President of the New York Heart Association.

Five Vice-Presidents were elected this year. They include Mrs. Alben Barkley, Washington; Bruce Barton, New York; T. Duckett Jones, M.D., New York; Robert L. King, M.D., Seattle; and Frederick K. Trask, Jr., New York. Grant Keehn, New York, was re-elected Treasurer.

Board of Directors

At a meeting of the Board of Directors, A. W. Robertson, Pittsburgh, was re-elected Chairman; William H. Bunn, M.D., Youngstown, O., was elected Secretary; and Harry E. Ungerleider, M.D., New York, was renamed Assistant Secretary.

New members of the Board of Directors, elected by the Assembly for a three year term are: Mrs. Alben Barkley, Washington; William A. Brumfield, Jr., M.D., Albany, N. Y.; P. O. Ferrel, Indianapolis; Irving B. Hexter, Cleveland; and Harold C. Lueth, M.D., Omaha.

Re-elected to the Board for three year terms were Herrman L. Blumgart, M.D., Boston; Louis N. Katz, M.D., Chicago; Grant Keehn, New York; H. M. Marvin, M.D., New Haven, Conn.; A. W. Robertson, Pittsburgh; Frederick K. Trask, Jr., New York; Maurice B. Visscher, M.D., Minneapolis; Robert W. Wilkins, M.D., Boston; and Irving S. Wright, M.D., New York.

New members elected to the Board for a one year term include: Bruce Barton, New York; John D. Brundage, Newark, N. J.; W. Montague Cobb, M.D., Washington: D. H. Griswold, Chattanooga; A. J. Hayes, Washington; David J. McDonald, Pittsburgh; Michel Puyans, New York; and J. Ross Veal, M.D., Washington.

Board members chosen to represent the Scientific Council and its component sections and councils include: Alva Bradley, Cleveland; J. Scott Butterworth, M.D., New York; Thomas M. Durant, M.D., Philadelphia; T. Duckett Jones, M.D., New York; R. Bruce League, M.D., Atlanta; Hugh McCulloch, M.D., Chicago; Hugh Montgomery, M.D., Philadelphia; Henry A. Schroeder, M.D., St. Louis; John R. Smith, M.D., St. Louis; Howard B. Sprague, M.D., Boston; Robert D. Taylor, M.D., Cleveland; and Leigh Willard, New York.

Scientific Council

Dr. Howard B. Sprague, Boston has become Chairman of the Scientific Council. Frank N. Wilson, M.D., Ann Arbor, Mich., has been renamed Vice-Chairman, and Lowell A. Rantz, M.D., San Francisco, has been reelected Secretary. Members of the Executive Committee elected to serve until 1954 include Eugene B. Ferris, Jr., M.D., Cincinnati; William B. Bean, M.D., Iowa City; and Arthur C. de Graff, M.D., New York.

Francis C. Wood, M.D., Philadelphia, has been chosen Chairman of the Research Committee. Other members elected are Robert H. Bayley, M.D., Oklahoma City; Howard B. Burchell, M.D., Rochester, Minn.; Jefferson M. Crismon, Stanford, Calif.; and Charles A. R. Connor, M.D., New York.

Editorial Board

The following have been elected to the Editorial Board of *Circulation* to serve until 1956: E. Cowles Andrus, M.D., Baltimore; George E. Burch, M.D., New Orleans; Howard B. Burchell, M.D., Rochester, Minn.; J. Russell Elkinton, M.D., Philadelphia; Hans H. Hecht, M.D., Salt Lake City, and Harold D. Green, M.D., Winston-Salem, N. C.

GOLD HEART AWARDS

Gold Heart Awards for outstanding contributions to cardiovascular medicine or the Association were presented at the Annual Dinner in Atlantic City in June to Drs. James B. Herrick, Frank N. Wilson, and H. M. Marvin. Dr. Herrick, who is emeritus Professor of Medicine at the University of Chicago and the University of Illinois, was honored for his work in the field of coronary thrombosis with myocardial infarction. Dr. Wilson, Professor of Medicine at the University of Michigan, received the Award for his investigations in the field of electrocardiography. Both Dr. Herrick and Dr. Wilson received commendation for their research last year in special issues of *Circulation*. Dr. Marvin received the Gold Heart for his many years of

service to the Association, including the Presidency in 1949–50, and his contribution to the popular literature of cardiovascular diseases as co-author and editor of "You and Your Heart."

Arthur H. Motley, President and Publisher of Parade Magazine, Sunday picture newspaper supplement, addressed the dinner on "The Responsibility of the Press in Reporting Advances in Medical Science."

WEST VIRGINIA FELLOWSHIP

The West Virginia Heart Association has contributed $2,500 to the national research fund of the American Heart Association for the support of a Fellowship. The Association plans to make the grant renewable annually, and in future years preference will be given to an applicant who either is a native of West Virginia or has received some of his training in that state.

CARDIAC-IN-INDUSTRY COMMITTEE

The Joint Committee on the Cardiac-In-Industry has held its first meeting in New York to consider problems of education, re-research, rehabilitation and employability of workers with heart disease. Certain recommendations have been made and these have been referred to the appropriate channels in the Association.

The Committee was set up by the Public Health Advisory Committee and the Scientific Council. Its Chairman is Dr. Leonard J. Goldwater, Professor of Industrial Hygiene, Columbia University School of Public Health, New York. Other members are Rufus B. Crain, M.D., Medical Director, Eastman Kodak Company, Rochester, N. Y.; Edward M. Kline, M.D., Medical Consultant, General Electric Company, Cleveland; Leo Price, M.D., Director, Union Health Center, International Ladies' Garment Workers' Union, New York; Brandt F. Steele, M.D., internist and psychiatrist, Philadelphia; James V. Warren, M.D., physiologist, Emory University School of Medicine, Atlanta.

HEART MODELS

An agreement has been concluded with Abram Belski, sculptor at New York Medical College, Department of Anatomy, for the construction of ten life-size models of the human heart. Three of the models will represent normal types and seven will show pathologic conditions. The models made of latex rubber will be sold by the Association to physicians and medical schools and will replace the plaster models previously available.

NUTRITION COMMITTEE

Dr. Frederick J. Stare, Chairman of the Department of Nutrition at Harvard University School of Public Health, has accepted the chairmanship of a Committee which will prepare a booklet on nutrition and heart disease, with particular reference to appropriate diets. The members of the Committee include Conrad J. Elvehjem, Ph.D., Dean, Graduate School, University of Wisconsin, Madison; Eugene B. Ferris, Jr., M.D., Assistant Director, Department of Internal Medicine, Cincinnati General Hospital; Mrs. Reena Roberts Hasker, Instructor in Nutrition, Harvard University School of Public Health; Kenneth G. Kohlstaedt, M.D., Lilly Clinic, Indianapolis General Hospital; Irvine H. Page, M.D., Director of Research, Cleveland Clinic Foundation; Louis Leiter, M.D., Clinical Professor of Medicine, Columbia University, New York; Arthur J. Merrill, M.D., Associate Professor of Medicine, Emory University Medical School, Atlanta.

The Committee will broaden the scope of the "Cookbook for Low Sodium Diet" recently issued by the Massachusetts Heart Association to include general information about nutrition as well as low sodium, obesity, and low cholesterol diets.

The completed booklet will be distributed nationally by the American Heart Association.

WYETH GRANT

Wyeth Incorporated, Philadelphia pharmaceutical firm, has renewed a grant of $3,600 to the Publication Fund of the Association. This grant permits the use of color in illustrations in *Circulation*.